Evaluation of stereochemically dense morpholine-based scaffolds as proline surrogates in β-turn peptides†

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Four peptides differing for the structure of the new morpholine-based heterocyclic compound acting as a turn inducer were synthesized in solution phase, and the conformational preferences were assessed by means of NMR analysis. All spectroscopic data revealed an adaptive behaviour of the turn peptides in generating turn conformations stabilized by intramolecular hydrogen-bonds, despite the conformational changes of the turn inducer. Thus, this study suggests the possibility of functionalizing morpholine-containing β-turn peptides with no significant loss of the secondary framework. The 3,4-dihydro-2*H*-[1,4]oxazine-containing peptide showed a more compact structure stabilized by an additional γ-turn-forming hydrogen-bond experienced by the Gly amide proton.

Introduction

β-Turns play a crucial role in proteins and bioactive peptides, due to their ability to induce folding and generate compact structures.[1,2] They are often involved in molecular recognition processes,[3] together with γ-turns, which are considered rare turns able to reverse the chain direction.[4,5] β-Turns consist of a tetrapeptide sequence in a non-helical region in which the chain direction is reversed, and are often stabilized by an intramolecular hydrogen-bond between the carbonyl oxygen of the first residue (*i*) and the amide proton of the fourth one (*i*+3) (Fig. 1, left), thus forming a ten-membered pseudo-cycle. Many β- and γ-turn mimetics have been designed so far. The majority of them are based on the replacement of the *i*+1–*i*+2 central dipeptidic sequence of the turn with dipeptide isosteres, able to preserve the intramolecularly hydrogen-bonded ten-membered pseudo-cycle.[6–18] Also, β-turn structures have been recently proposed by several research groups as effective organocatalysts in a variety of transformations,[19] suggesting the development of turn mimetics as an opportunity for advances in catalysis and organic synthesis.

A strong interest is focused on proline mimetics (Fig. 1, right), as among the naturally occurring amino acids proline is often observed at the *i*+1 position of β-turn structures, generating a *trans* amide bond with the preceding amino acid at position *i*. Also, the unnatural D-enantiomer of proline favors antiparallel β-sheet formation *via* type I′/II′ β-turns.[20]

We recently reported a new method for the synthesis of enantiopure Fmoc-protected morpholine-3-carboxylic acid from dimethoxyacetaldehyde and serine methyl ester through a short and practical synthetic route,[21] and the conformational analysis

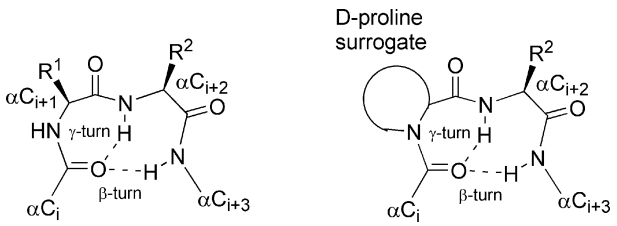

Fig. 1 β-/γ-turn structures (left), and modified motifs using cyclic templates at *i*+1 position (right).

of peptides containing morpholine-3-carboxylic acid (Mor) as an unexplored proline surrogate.[22] The relevance of such a secondary amino acid in medicinal chemistry is remarkable, as morpholine-3-carboxylic acid is present in several bioactive molecules, such as TACE (TNF-α converting enzyme),[23] MMP (matrix metalloproteinase), and TNF (tumour necrosis factor) inhibitors,[24] and a potent orally active VLA-4 antagonist.[25] With the aim of gaining information for the design of turn peptides containing the morpholine nucleus, we decided to study the conformational role of more complex morpholines. Specifically, in this paper we report the conformational analysis of model heterochiral peptides containing new stereochemically dense morpholine-carboxylic acids at position *i*+1. Such a study was conceived to assess the role of the morpholine stereochemistry (Fig. 2, structures **I–III**) and the change of hybridization as found in the 3,4-dihydro-2*H*-[1,4]oxazine nucleus (Dox) (Fig. 2, structure **IV**) in determining the conformational preferences of the peptide.[26]

Results and discussion

Synthesis

The synthesis of the new morpholine-3-carboxylic acids embedded in peptides **I–IV** was developed using threonine as the starting material. Morpholine acetal **1** was prepared, according to a previously reported procedure, as a mixture of two epimeric acetals in an approximately 1 : 1 ratio (Scheme 1).[27]

Fmoc protection of **1** allowed a simple chromatographic separation of the two diastereomeric acetals **2** and **3**. Subsequent basic

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† Electronic supplementary information (ESI) available: Experimental procedures and characterization data for H-Gly-D-Leu-D-Val-OMe, **9a–c** and **10a–c**, nOe spectrum of H-6 of **1a**, ^{1}H NMR, ^{13}C NMR and ROESY data of **I–IV**, copies of ^{1}H NMR and ^{13}C NMR spectra of compounds **1–11**, copies of ^{1}H, TOCSY, ROESY and gHSQC spectra of peptides **I–IV**, and computational data for model scaffolds of Fig. 6. See DOI: 10.1039/b913444a

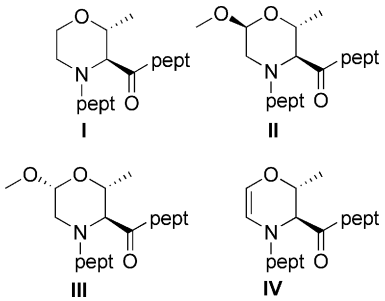
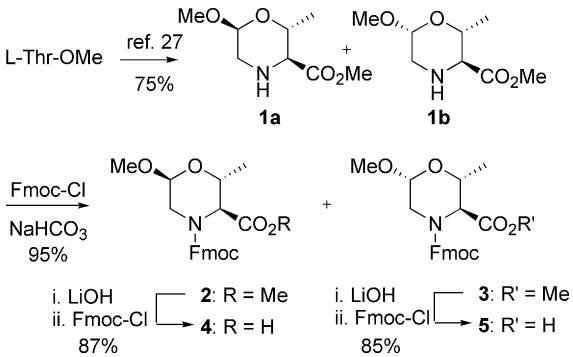
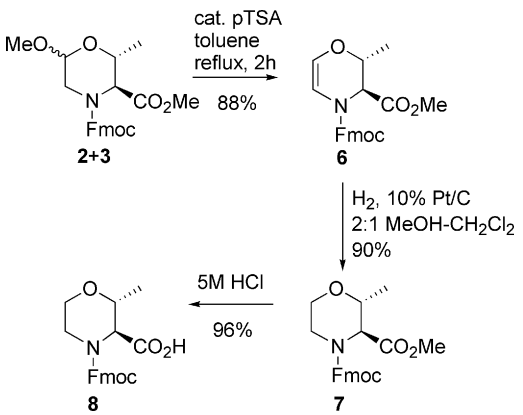


Fig. 2 Selected scaffolds as turn inducers.



Scheme 1 Preparation of Fmoc-2-methyl-6-OMe-Mor-OH derivatives **4** and **5**.

hydrolysis followed by *in situ* reprotection of the nitrogen group lead to the isolation of acids **4** and **5** in high yield and without epimerization at the acetalic carbon atom. Fmoc-amino acid **8** was prepared by refluxing a mixture of acetals **2** and **3** in toluene in the presence of a catalytic amount of pTSA in order to obtain the corresponding methyl Fmoc-3,4-dihydro-2*H*-[1,4]-oxazine-3-carboxylate **6**, which was successively hydrogenated under Pt/C catalysis and hydrolyzed with 5M HCl, to give the final Fmoc-amino acid **8** (Scheme 2). The stereochemical assignment of the two acetals was achieved by nOe experiments on both unprotected 3-carbomethoxy-6-methoxy-2-methyl-morpholines **1a** and **1b**. Specifically, a strong nOe interaction between H-6 and H-2 protons of **1b** (Fig. 3) revealed a *cis* configuration of the methoxy and methyl substituents at C-6 and C-2, respectively, thus enabling the unambiguous assignment of the stereochemistry of compound **1b**.[28]



Scheme 2 Synthesis of Fmoc-2-methyl-Mor-OH **8**.

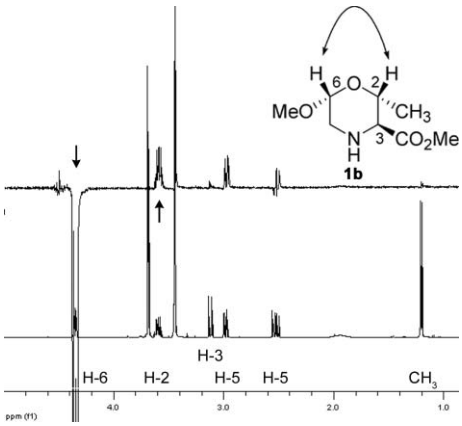


Fig. 3 Diagnostic nOe peaks between the irradiated proton H-6 at 4.35 ppm (down arrow) and H-2 at 3.60 ppm (up arrow) of compound **1b**.

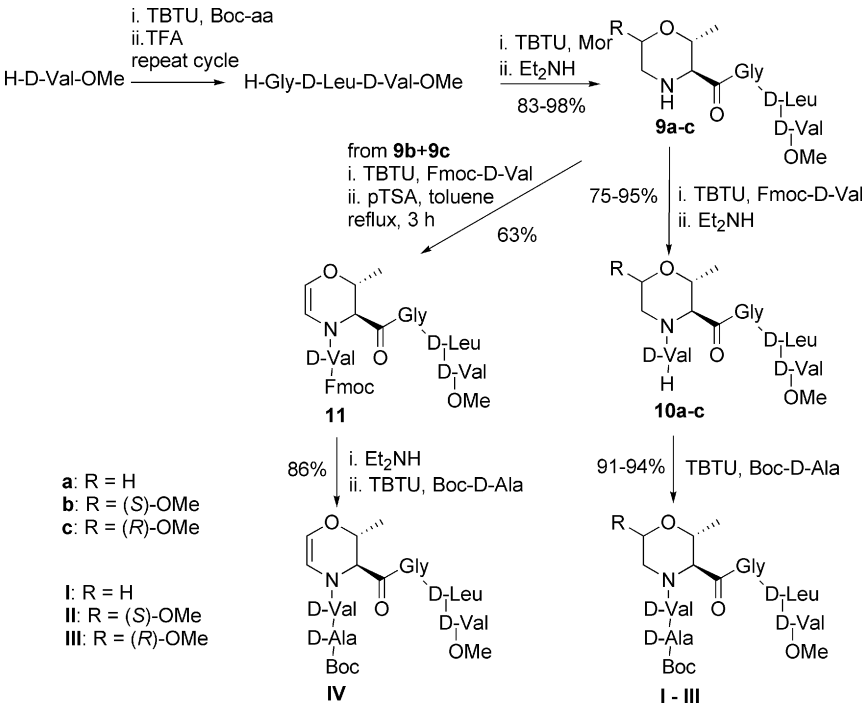
The synthesis of the heterochiral peptides **I–III** of general sequence Boc-D-Ala-D-Val-(Mor)-Gly-D-Leu-D-Val-OMe was achieved by solution-phase peptide synthesis using both the Boc and the Fmoc strategy, TBTU as the coupling agent, and diethylamine as the Fmoc-deblocking agent (Scheme 3). The H-Gly-D-Leu-D-Val-OMe sequence was prepared and coupled with the three different Fmoc-morpholine derivatives **4**, **5** and **8**. Finally, the corresponding peptides **II**, **III** and **I** were achieved by sequential coupling of Fmoc-D-Val and Boc-D-Ala. Peptide **IV** was prepared by subjecting the intermediate peptides **10b–10c**, containing the 6-OMe-morpholine scaffold, to acid-catalyzed double bond formation to give the corresponding (Dox)-containing peptide **11**, followed by Fmoc deprotection and coupling of the terminal Boc-D-Ala residue.

The title peptides were purified by flash chromatography and obtained pure with an overall yield ranging from 44–77%.

Conformational analysis

The conformational analysis of the four model peptides **I–IV**, containing morpholine or 3,4-dihydro-2*H*-[1,4]oxazine derivatives, was assessed by 1D- and 2D-NMR experiments.

The behaviour of amide protons was studied in $CDCl_3$, a relatively non-polar solvent, which is well-suited for evaluating the intrinsic conformational features of small oligoamides, and CD_3CN, a moderate hydrogen-bond acceptor with enhanced solvating properties, useful to test the strength of intramolecular hydrogen-bonds. Specifically, in a relatively non-polar solvent, intramolecular hydrogen bonds provide a driving force for folding, whereas in aqueous solution, conventional peptides comprised of 2–6 residues do not generally display significant populations of folded conformers, as a consequence of limited noncovalent driving force for nucleation of a compact conformation. Since solvation forces are expected to have relatively little influence on the intrinsic folding propensity, the insights gained from analysis in organic solvents should apply to aqueous solution as well.[29] The hydrogen-bonding preferences were studied by comparing amide proton chemical shifts in different solvents, and as a function of increasing quantities of DMSO-d_6 in $CDCl_3$ solutions. Details about three-dimensional structures were elucidated through ^1H and ^{13}C 2D experiments (gHSQC, TOCSY and ROESY).



Scheme 3 Synthesis of peptides **I–IV**.

1D-NMR data of all peptides **I–III** showed a single set of signals, indicating the existence of a unique rotamer around the Mor-D-Val amide bond. ROESY data showed sequential ROESY peaks between Val H-α and H-5 protons of the morpholine nucleus, which allowed the assignment of this structure as the *trans* isomer. In the case of peptide **IV**, a 4 : 1 mixture of rotamers was found in both 1D-NMR recorded in $CDCl_3$ and CD_3CN, suggesting the flatter oxazine nucleus to stabilize a minor amount of the *cis* conformation around the Dox-D-Val amide bond.

The analysis of the chemical shifts of amide protons suggested a modulation of the intramolecular hydrogen-bonding as a function of the scaffold at *i*+1 position of the turn peptide. In all the four peptides, D-Ala NH did not show any conservation of the chemical shift value changing from $CDCl_3$ to CD_3CN as solvents, suggesting a typical non-hydrogen-bonded state for such protons (Table 1). Similarly, the D-Val_2 amide proton experienced deviations higher than 0.3 ppm, and only 0.18 ppm in peptide **IV**, indicating a low hydrogen-bonding character. The conservation of the chemical shift of Leu NH upon changing the solvent indicated a marked tendency to establish intramolecular hydrogen-bonds. Val_1 amide proton showed a negative $\Delta\delta$ value when shifting from CD_3CN to $CDCl_3$, indicating a stronger hydrogen-bonding character in the less interacting solvent, as expected. These data proved the existence of β-hairpin structures stabilized by two intramolecular hydrogen bonds in analogy with the β-sheet structures induced by the central D-Pro-Gly dipeptidic sequences.[19] The evidence of Leu and Val_1 amide protons as the most deshielded protons in peptides **I–III** also confirmed this general outcome. The behaviour of Gly NH was strictly dependent on the cyclic amino acid at the *i*+1 position, as by moving from Mor to Dox, a marked stabilization of an intramolecular hydrogen-bond experienced by such amide protons was observed. In fact, a downfield shift from 6.72–7.08 to 7.50 ppm was observed in $CDCl_3$, and more interestingly a conservation of the chemical shift was observed in peptide **IV**, as shown in Table 1, compared with $\Delta\delta$ values ranging from 0.27 to 0.53 corresponding to Gly NH of compounds **I–III**.

Table 1 $\Delta\delta$ of amide protons chemical shifts in $CDCl_3$ and CD_3CN

Peptide	Solvent	D-Ala	D-Val_1	Gly	D-Leu	D-Val_2
I	$CDCl_3$	5.32	7.65	7.08	7.11	6.69
	CD_3CN	5.71	7.37	7.35	7.13	7.01
	$\Delta\delta$ NH[a]	0.39	–0.28	0.27	0.02	0.32
II	$CDCl_3$	5.29	7.79	6.79	7.16	6.71
	CD_3CN	5.63	7.60	7.30	7.28	7.02
	$\Delta\delta$ NH[a]	0.34	–0.19	0.51	0.12	0.31
III	$CDCl_3$	5.32	7.68	6.72	7.40	6.62
	CD_3CN	5.67	7.55	7.25	7.40	7.03
	$\Delta\delta$ NH[a]	0.35	–0.13	0.53	0	0.41
IV	$CDCl_3$	5.27	7.50	7.50	6.53	6.74
	CD_3CN	5.63	7.23	7.47	6.61	6.92
	$\Delta\delta$ NH[a]	0.46	–0.27	–0.03	0.08	0.18

[a] Values are obtained by subtracting δ NH($CDCl_3$) to δ NH(CD_3CN).

1D Experiments in the presence of increasing quantities of DMSO-d_6 suggested a smooth modulation of the conformational profile as a function of the heterocyclic amino acid at position *i*+1 of the turn (Fig. 4). The reference hexapeptide containing proline was also subjected to this experiment so as to directly compare the behaviour of the morpholine-based scaffolds to proline.[30] Peptide **I** showed a strong conservation of the chemical shift value of Val_1 NH, and a $\Delta\delta$ of 0.3 ppm for Leu NH. Together with the corresponding chemical shift values of 7.65 and 7.11 ppm, these data suggested that the existence of a β-hairpin structure stabilized two intramolecular hydrogen-bonds, in analogy with the proline-containing reference peptide. On the contrary, Val_2

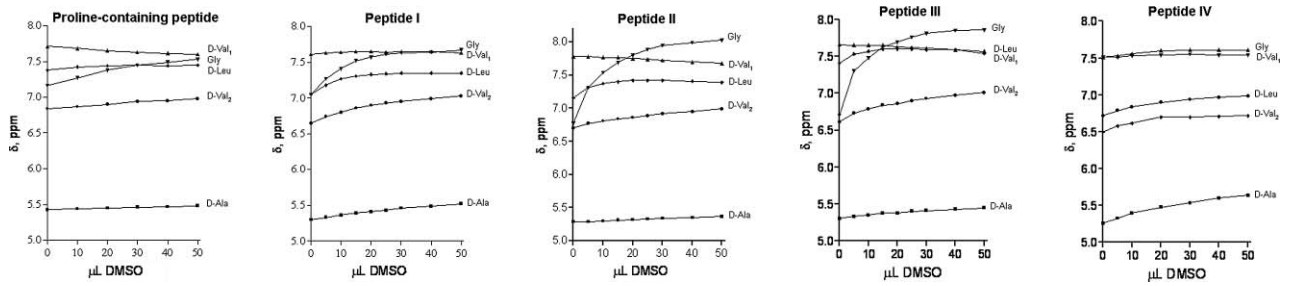


Fig. 4 ^{1}H NMR DMSO-d_6 titrations of compounds **I–IV** and of reference peptide containing proline in $CDCl_3$.

showed a marked chemical shift difference with respect to Val_1 NH, and a higher $\Delta\delta$ deviation upon addition of deuterated DMSO, suggesting a non-hydrogen-bonding state for such protons. Gly amide proton displayed a larger dependence upon DMSO-d_6 addition than observed in the reference proline-peptide, and also D-Ala urethane proton suggested no involvement in hydrogen-bonding. Peptide **II**, containing the (6*S*)-methoxy-2-methyl-Mor scaffold, showed a similar profile to peptide **I**, and an enhanced solvent effect to the chemical shift variation of Gly amide proton (Fig. 4). Also, a small upfield shift of the hydrogen-bonded Val_1 NH indicated a modulation of the conformational profile as a function of solvent polarity. Peptide **III**, containing (6*R*)-methoxy-2-methyl-Mor as the D-proline surrogate showed a more compact doubly hydrogen-bonded β-hairpin structure as a consequence of a downfield shift, and small $\Delta\delta$ values for Val_1 and Leu amide protons in analogy with the reference proline-peptide. Similarly to peptide **II**, Gly NH showed a dramatic dependence on the chemical shift to % amounts of deuterated DMSO, suggesting a complete solvent-exposed orientation of such amide protons in the rigid conformation of **III** compared to the reference proline-peptide.

Peptide **IV**, containing the Dox scaffold, indicated a change in the hydrogen-bonding pattern of the peptide when moving from Mor to the flatter dihydro-1,4-oxazine nucleus (Fig. 4). In fact, Gly NH showed the highest chemical shift value compared to peptides **I–III** and lowest chemical shift deviation as a function of DMSO-d_6 additions, suggesting that this proton engaged in a strong intramolecular hydrogen-bond, in analogy with Val_1 NH. Conversely, Leu NH displayed the lowest chemical shift value compared to peptides **I–III** indicating a lowered capability to experience intramolecular hydrogen-bonds. These data proved peptide **IV** to fold into γ-turn and β-hairpin-like structures stabilized by 7- and 14-membered ring intramolecular hydrogen-bonds, established by Gly NH and Val_1 NH, respectively. Also, the analysis of amide proton chemical shifts may suggest a role of the methyl at C-2 and of the ring size to prevent any hydrogen-bonding by Gly NH proton, and a smooth modulation of the hydrogen-bonding pattern exerted by tuning the stereochemistry at C-6.

TOCSY and ROESY data for all the four peptides were carried out in the more interactive CD_3CN solvent (see Table S3 in the ESI†), and significant ROESY peaks are shown in Fig. 5. Compounds **I–III** displayed the diagnostic through-space correlations typical of β-turn peptides. A ROESY cross-peak between Gly NH and H-2 of the Mor nucleus was found in all the three peptides and suggested a type II′ β-turn.

Peptides **II** and **III** showed the key Gly H-α and Leu NH correlation, and compound **III** displayed the additional ROESY

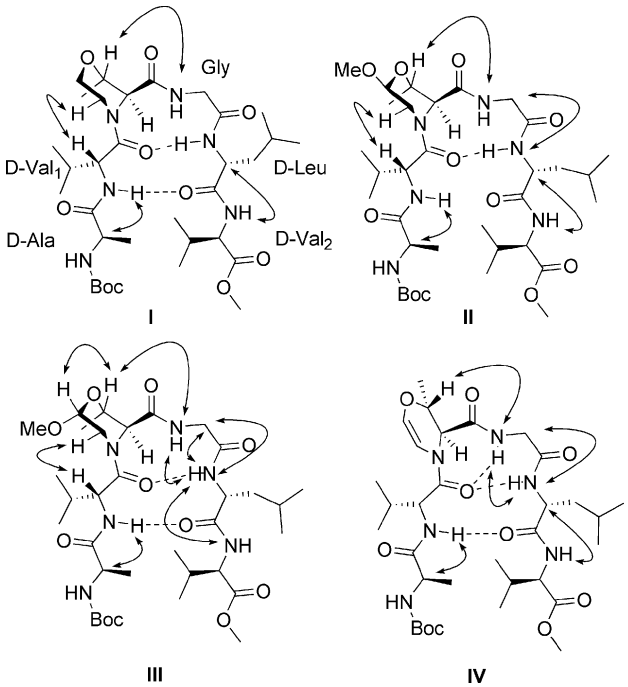


Fig. 5 Reverse turn conformations for peptides **I–IV**: the arrows indicate significant ROESY cross-peaks.

correlation between Gly NH and Leu NH, giving evidence of this compound as the most stable reverse turn conformation. ROESY data for compound **IV** (Fig. 5, bottom right) confirmed the existence of a γ-turn stabilized by a hydrogen-bond between Gly NH and the Val_1 carbonyl group, as evinced by Gly NH/H-2 and Leu NH/Gly NH ROESY cross-peaks. These data contributed to assign a reverse turn conformation for compound **IV** stabilized by 7- and 14-membered ring hydrogen-bonds as a consequence of the modulation of the conformational profile of the peptide by the flatter dihydro-oxazine scaffold. The ROESY interaction between H-2 and Gly NH suggested the methyl group at C-2 to contain all the peptides in similar well-defined turn conformations. The modulation of the secondary structure by the stereochemistry at C-6 was less significant, indicating the axial orientation the most favourable to generate a turn peptide, as observed in peptide **III**.

With the aim of giving more insight into the role of the proline surrogates taken into account herein on the overall conformation of the peptide turn, molecular modeling calculations were carried out on model morpholine and dihydro-[1,4]oxazine scaffolds to evaluate the preference for axial or equatorial orientation of the substituents at C-2 and C-3 carbon atoms of the scaffolds. Thus,

the four *N*-acetyl-scaffold-methylamides possessing a *trans* geometry at the nitrogen atom were subjected to AM1 semi-empirical calculations to optimize the global minimum conformer. The geometry of the most abundant minimum energy conformer was successively subjected to *ab initio* single point energy calculation at the 3-21G*/HF level of quantum chemical theory. In all the four model structures, the energy difference in kcal mol^{-1} between the axial and equatorial conformations was computed and compared so as to establish the most stable structure.

Interestingly, the model structures corresponding to proline surrogates included in peptides **I** and **III** (Fig. 6, top left and bottom left, respectively) resulted in the most stable conformations having the substituents at C-2 and C-3 in equatorial orientation, as demonstrated by the energy difference of +1.0 and +6.6 kcal mol^{-1}, respectively, whereas the model structures corresponding to proline surrogates of peptides **II** and **IV** (Fig. 6, top right and bottom right, respectively) resulted in the most stable conformations possessing the same substituents in axial orientation (ΔE_{ax-eq} of –6.2 and –6.9 kcal mol^{-1}, respectively). The evidence of a similar conformational profile for 2-methyl-Mor and (6*R*)-methoxy-2-methyl-Mor was in agreement with the most stable β-hairpin conformations displayed by the corresponding peptides **I** and **III**, as shown by NMR data, thus indicating a role of the orientation of such substituents on the conformational effect of the proline surrogate in nucleating well-defined conformational arrangements. Also, the planar arrangement of the model structure of the dihydro-[1,4]oxazine nucleus (Fig. 6, bottom right) may explain the marked tendency of the Gly amide proton to experience a strong intramolecular hydrogen-bond with D-Val_i carbonyl group.

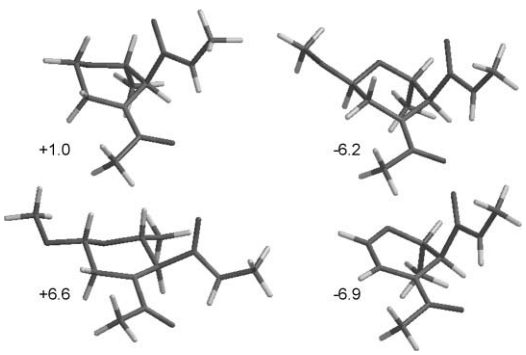


Fig. 6 Minimum energy conformations of model structures containing the four scaffolds employed in peptide synthesis: 4-acetyl-2-Me-Mor-3-methylamide (top left); 4-acetyl-2-Me-(6*S*)-OMe-Mor-3-methylamide (top right); 4-acetyl-2-Me-(6*R*)-OMe-Mor-3-methylamide (bottom left); 4-acetyl-2-Me-3,4-dihydro-2*H*-[1,4]oxazine-3-methylamide (bottom right). The values under each structure represent the energy difference in kcal mol^{-1} between the axial and the equatorial conformations, where the axial structure is referred to the orientation of the substituents at C-2 and C-3.

Conclusions

The combination of threonine and dimethoxyacetaldehyde derivatives as building blocks allowed the generation of four new cyclic Fmoc-protected amino acids, and their insertion in model β-turn peptides was demonstrated by solution-phase peptide synthesis. The conformational analysis by 1D and 2D-NMR of four heterochiral peptides containing the four new cyclic amino acids as proline surrogates, acting as turn inducers, revealed an adaptive behaviour of the turn peptides in generating turn conformations stabilized by intramolecular hydrogen-bonds irrespective of the conformational changes of the heterocyclic structure imposed by a different hybridization of the atoms or the stereochemical arrangements of the substituents. More detailed analysis, supported by molecular modeling calculations, indicated a smooth modulation of the turn propensity by the orientation of the substituents on the cyclic amino acid, although the reverse turn conformation was always maintained, thus suggesting the possibility of functionalizing morpholine-containing β-turn peptides with no significant loss of the secondary framework. The 3,4-dihydro-2*H*-[1,4]oxazine-containing peptide showed a more compact structure stabilized by an additional γ-turn-forming hydrogen-bond experienced by the Gly amide proton, thus indicating the change in the hybridization from Mor to Dox as the main source of conformational changes in β-turn peptides.

These results are of importance as a guide for the design of reverse turn mimetics with morpholine and dihydro-[1,4]oxazine derivatives embedded in the peptide backbone. Also, the modulation of the substituent pattern in the morpholine nucleus and its recognised importance in medicinal chemistry may constitute an opportunity for the generation of new reverse turn peptidomimetics containing privileged structures.

Experimental

General

Chromatographic separations were performed on silica gel (Kieselgel 60, Merck) using flash-column techniques; R_f values refer to TLC carried out on 25 mm silica gel plates (Merck F_{254}) with the same eluent as indicated for column chromatography. ESI mass spectra were carried out on an ion-trap double quadrupole mass spectrometer using electrospray (ES^+) ionization techniques, and a normalized collision energy within the range of 25–32 eV for MSMS experiments.

NMR methods

^{1}H NMR spectra were recorded with a Varian INOVA NMR spectrometer operating at 400 MHz , and ^{13}C NMR spectra with a Varian Gemini operating at 50 MHz. All NMR spectra are referenced to residual protonated NMR solvent. The spectra for the conformational analysis of peptides **I–IV** were obtained in 3–5 mM $CDCl_3$ or CD_3CN solutions where aggregation was not significant. Proton signals were assigned *via* TOCSY spectra, and ROESY spectra provided the data used in the conformational analyses. TOCSY spectra were recorded with a mixing time of 80 ms, 2048 points in *t*1, 256 points in *t*2, and 8 scans per *t*2 increment. ROESY spectra were recorded with a mixing time of 500 ms, a similar number of *t*1 and *t*2 points unless otherwise stated, and 32 per *t*2 increment. ^{13}C NMR data were assigned *via* gHSQC spectra. NOESY1D experiments on **1a** and **1b** were carried out with a mixing time of 500 ms.

Computational methods

Calculations on the model compounds Ac-2-Me-6-H/OMe-Mor-NHEt and Ac-2-Me-Dox-NHEt were performed using SPARTAN version 5.147 running on a SGI IRIX 6.5 workstation. Conformational searches were carried out using Monte Carlo method within MMFF94 force field, and the AM1 semiempirical method was used to optimize the global minimum conformer.[31] The geometry of the most abundant minimum energy conformer was successively subjected to *ab initio* single point calculation of the electronic properties at the 3-21G*/HF level of quantum chemical theory.[31]

(2*R*,3*S*,6*R*/*S*)-6-Methoxy-2-methyl-morpholine-3-carboxylic acid methyl ester (1). Compound **1** was prepared as reported in ref. 27. The product was directly used without further purification for the protection step. An analytical quantity of the mixture was purified by flash chromatography to enable the assignment of the stereochemistry of the two diastereomeric acetals. **(2*R*,3*S*,6*S*)-1a**: δ_H (400 MHz, $CDCl_3$) 4.44 (s, 1 H, H-6), 3.85 (dq, $J = 9.7$, 6.2 Hz, 1 H, H-2), 3.70 (s, 3 H, CO_2CH_3), 3.36 (s, 3 H, OCH_3), 3.25 (d, $J = 10.0$ Hz, 1 H, H-3), 2.89 (s, 2 H, H-5), 2.1 (br, 1 H, NH), 1.13 (d, $J = 6.0$ Hz, 3 H, CH_3); δ_C (100 MHz, $CDCl_3$) 171.3 (s, CO_2Me), 95.7 (d, C-6), 65.6, 63.6, 54.4 (q, OCH_3), 51.9 (q, OCH_3), 47.2 (t, C-5), 18.1 (q, CH_3). **(2*R*,3*S*,6*R*)-1b**: δ_H (400 MHz, $CDCl_3$) 4.35 (dd, $J = 8.9$, 2.5 Hz, 1 H, H-6), 3.68 (s, 3 H, OCH_3), 3.60 (dq, $J = 9.0$, 6.2 Hz, 1 H, H-2), 3.44 (s, 3 H, OCH_3), 3.12 (d, $J = 9.0$ Hz, 1 H, H-3), 2.98 (dd, $J = 12.7$, 2.5 Hz, 1 H, H-5), 2.52 (d, $J = 12.7$, 8.9 Hz, 1 H, H-5), 1.20 (d, $J = 6.3$ Hz, 3 H, CH_3). δ_C (100 MHz, $CDCl_3$) 171.4 (s, CO_2Me), 100.6 (d, C-6), 73.7, 62.8, 56.0 (q, OCH_3), 52.0 (q, OCH_3), 47.9 (C-5), 18.1 (q, CH_3).

(2*R*,3*S*,6*R*/*S*)-6-Methoxy-2-methyl-morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester (2 and 3). The crude cyclic acetal **1** was dissolved in H_2O (13 mL) and $NaHCO_3$ (1.33 g, 15.9 mmol) was added. The mixture was stirred until complete dissolution of the salt and then dioxane (20 mL) was added. The flask was cooled at 0 °C with an ice bath and solid Fmoc-Cl (1.17 g, 4.55 mmol) was added in portions. After 10 min the ice bath was removed, and the reaction mixture was stirred 16 h at room temperature, then EtOAc (30 mL) and water (20 mL) were added. The aqueous layer was discarded and the organic phase was washed with 5% citric acid, brine and dried over Na_2SO_4. The solvents were removed under reduced pressure and the crude material was purified by flash column chromatography (hexanes–AcOEt 6 : 1 to 4 : 1) to provide pure **2** (374 mg, 21%), pure **3** (461 mg, 26%) and 943 mg of mixed fractions. The combined yield of both diastereoisomer **2** and **3** was 1.778 g (95%). **(2*R*,3*S*,6*S*)-2**: (Found: C, 67.31; H, 6.21; N, 3.18. $C_{23}H_{25}NO_6$ requires C, 67.14; H, 6.12; N, 3.40%). $[\alpha]_D^{26}$ −3.3 (*c* 1, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 1 : 1 mixture of rotamers 7.76 (d, $J = 7.6$ Hz, 2 H, Fmoc CH), 7.55 (q, $J = 6.4$ Hz, 2 H, Fmoc CH), 7.40 (t, $J = 7.6$ Hz, 2 H, Fmoc CH), 7.31 (m, 2 H, Fmoc CH), 4.81 (t, $J = 7.6$ Hz, 0.5 H, H-6), 4.71 (t, $J = 7.6$ Hz, 0.5 H, H-6), 4.52-4.38 (m, 2 H), 4.38-4.17 (m, 2 H), 4.12-3.96 (m, 2 H), 3.74 (s, 1.5 H, CO_2CH_3), 3.67 (s, 1.5 H, CO_2CH_3), 3.41 (s, 3 H, OCH_3), 3.14 (dd, $J = 13.6$ Hz, 0.5 H), 3.02 (dd, $J = 13.6$ Hz, 0.5 H), 1.38 (t, $J = 6.8$ Hz, 3 H, CH_3); δ_C (50 MHz, $CDCl_3$) 1 : 1 mixture of rotamers 170.1 (s, CO_2Me), 155.1 (s, NCO), 143.6 (s, 2 C, Fmoc), 141.0 (s, 2 C, Fmoc), 127.5 (d, 2 C, Fmoc), 126.9 (d, 2 C, Fmoc), 124.7 (d, 2 C, Fmoc), 119.8 (d, 2 C, Fmoc), 97.0 and 96.4 (d, C-6), 67.8 (t, CH_2OC), 64.9 and 64.4 (d, C-2), 61.5 and 61.0 (d, C-3), 55.3 (q, OCH_3), 52.3 (q, OCH_3), 47.1 (d, $CHCH_2OC$), 42.9 and 42.0 (t, C-5), 19.1 (q, CH_3). ESI-MSMS *m*/*z* 434.30 (M^++Na, 100). **(2*R*,3*S*,6*R*)-3**: (Found: C, 67.35; H, 6.23; N, 3.23. $C_{23}H_{25}NO_6$ requires C, 67.14; H, 6.12; N, 3.40%). $[\alpha]_D^{26}$ −81.4 (*c* 1, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) mixture of rotamers 7.76 (d, $J = 7.6$ Hz, 2H, Fmoc CH), 7.61 (m, 2 H, Fmoc CH), 7.40 (t, $J = 7.2$ Hz, 2 H), 7.31 (t, $J = 7.6$ Hz, 2 H, Fmoc CH), 4.67 (t, $J = 3.2$ Hz, 1 H, H-6), 4.48 (dd, $J = 10.4$, 7.2 Hz, 1 H), 4.42 (m, 4 H), 4.0-3.52 (m, 2 H), 3.77 (s, 3 H, CO_2CH_3), 3.46 (s, 3 H, OCH_3), 1.45 (br, 3 H, CH_3); δ_C (50 MHz, $CDCl_3$) mixture of rotamers 170.0 (s, CO_2Me), 156.0 (s, NCO), 143.5 and 143.4 (s, 2 C, Fmoc), 141.0 (s, 2 C, Fmoc), 127.4 (d, 2 C, Fmoc), 126.8 (d, 2 C, Fmoc), 124.8 (d, 2 C, Fmoc), 119.7 (d, 2 C, Fmoc), 96.7 (d, C-6), 69.1 (d, C-2), 67.9 (t, CH_2OC), 59.1 (d, C-3), 55.3 (q, OCH_3), 52.4 (q, OCH_3), 47.0 (d, $CHCH_2OC$), 44.3 (t, C-5), 20.2 (q, CH_3). ESI-MSMS *m*/*z* 434.28 (M^++Na, 100).

(2*R*,3*S*)-2-Methyl-2,3-dihydro-[1,4]oxazine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester (6). A solution of compounds **2** and **3** (736 mg, 1.79 mmol) in toluene (15 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (34 mg, 0.18 mmol) was placed in a single-necked round-bottomed flask equipped with a reflux condenser and a dropping funnel containing approximately 10 g of 4 Å molecular sieves. The mixture was refluxed for 2 h, then it was cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (hexanes–EtOAc 7 : 2, R_f 0.50) to yield compound **6** as a white foam (598 mg, 88%). (Found: C, 69.90; H, 5.65; N, 3.52. $C_{22}H_{21}NO_5$ requires C, 69.64; H, 5.58; N, 3.69%). $[\alpha]_D^{27}$ +6.3 (*c* 1.15, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3 : 2 mixture of rotamers 7.69 (t, $J = 8.8$ Hz, 2 H, Fmoc CH), 7.53 (dd, $J = 10.8$, 7.6 Hz, 1 H, Fmoc CH), 7.42 (d, $J = 8.0$ Hz, 1H, Fmoc CH), 7.33 (dd, $J = 14.4$, 7.2 Hz, 2 H, Fmoc CH), 7.27-7.20 (m, 2 H, Fmoc CH), 6.26 (d, $J = 4.8$ Hz, 0.33 H, H-6), 6.21 (d, $J = 4.8$ Hz, 0.66 H, H-6), 5.77 (d, $J = 4.8$ Hz, 0.33 H, H-5), 5.75 (d, $J = 4.8$ Hz, 0.66 H, H-5), 4.79 (qd, $J = 6.8$, 1.2 Hz, 0.66 H, H-2), 4.67 (qd, $J = 6.8$, 1.2 Hz, 0.33 H, H-2), 4.62 (s, 0.66 H), 4.95-4.62 (m, 1.33 H), 4.45-4.35 (m, 0.66 H), 4.27-4.23 (m, 1 H), 4.17 (t, $J = 6.4$ Hz, 0.33 H), 3.69 (s, 2 H, CO_2CH_3), 3.62 (s, 2 H, CO_2CH_3), 1.25 (d, $J = 6.8$ Hz, 2 H, CH_3), 1.15 (d, $J = 6.8$ Hz, 1 H, CH_3); δ_C (50 MHz, $CDCl_3$) mixture of rotamers 168.3 (s, CO_2Me), 152.7 and 151.9 (s, NCO), 143.5 and 143.2 (s, 2 C, Fmoc), 141.1 (s, 2 C, Fmoc), 127.6-124.5 (d, 7 C, Fmoc and C-6), 119.9 (d, 2 C, Fmoc), 104.5 and 104.0 (d, C-5), 69.8 and 69.2 (d, C-2), 68.3 and 67.8 (t, CH_2OC), 57.9 and 57.4 (d, C-3), 52.7 (q, OCH_3), 47.1 and 47.0 (d, $CHCH_2OC$) 17.3 (q). ESI-MSMS *m*/*z* 402.29 (M^++Na, 47), 315.04 (100).

(2*R*,3*S*)-2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester 3-methyl ester (7). Compound **6** (530 mg, 1.40 mmol) was dissolved in a 2 : 1 mixture of MeOH–CH_2Cl_2 (15 mL), and 10% Pt/C (63 mg) was added. The suspension was hydrogenated overnight at room temperature, and then filtered over Celite. The organic solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (hexanes–EtOAc 3 : 1, R_f 0.41) to yield pure **7** as a white foam (480 g, 90%). (Found: C, 69.30; H, 6.14; N, 3.59. $C_{22}H_{23}NO_5$ requires C, 69.28; H, 6.08; N, 3.67%). $[\alpha]_D^{23}$ −33.6 (*c* 1.1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) mixture of rotamers 7.76

(d, J = 7.6 Hz, 2 H, Fmoc CH), 7.56 (m, 2 H, Fmoc CH), 7.40 (t, J = 7.6 Hz, 2 H, Fmoc CH), 7.31 (t, J = 6.8 Hz, 2 H, Fmoc CH), 4.56-4.40 (m, 3 H), 4.40-4.00 (m, 2 H), 3.85-3.62 (m, 1 H), 3.75 (s, 3 H, CO_2CH_3), 3.65-3.20 (m, 2 H), 3.56 (d, J = 12.0 Hz, 1 H, H-5); 1.33 (d, J = 6.4, 3 H, CH_3); δ_C (50 MHz, $CDCl_3$) mixture of rotamers 169.8 (s, CO_2Me), 156.3 (s, NCO), 143.4 and 143.3 (s, 2 C, Fmoc), 141.0 (s, 2 C, Fmoc), 127.4 (d, 2 C, Fmoc), 126.8 (d, 2 C, Fmoc), 124.5 (d, 2 C, Fmoc), 119.7 (d, 2 C, Fmoc), 69.4 (d, C-2), 67.6 (t, CH_2OC), 58.6 (d and t, 2 C, C-3 and C-6), 52.4 (q, OCH_3), 47.1 (d, $CHCH_2OC$), 41.1 (t, C-5), 16.6 (q, CH_3). ESI-MSMS m/z 404.00 (M^++Na, 1), 372.51 (M^+–OMe+Na, 46), 317.20 (100).

(2*R*,3*S*)-2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester (8). Ester **7** (420 g, 1.1 mmol) was dissolved in dioxane (4 mL) and 5 M HCl (4 mL) was added. The reaction was refluxed for 18 h and then diluted with 5% Na_2CO_3 (30 mL). The resulting solution was washed with diethyl ether and then the aqueous layer was acidified to pH 1 with concentrated HCl and the organic phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure to yield compound **8** as a white solid (390 mg, 96%). (Found: C, 68.71; H, 5.83; N, 3.74. $C_{21}H_{21}NO_5$ requires C, 68.65; H, 5.76; N, 3.81%). $[\alpha]_D^{24}$ –26.7 (c 2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) mixture of rotamers 7.75 (d, J = 6.4 Hz, 2 H, Fmoc CH), 7.57 (m, 2 H, Fmoc CH), 7.39 (t, J = 6.8 Hz, 2 H, Fmoc CH), 6.62 (br, 1 H, CO_2H), 4.58-4.41 (m, 3 H), 4.40 (m, 0.5 H), 4.26 (m, 1 H), 4.11 (m, 0.5 H), 3.85-3.62 (m, 1.5 H), 3.62-3.45 (m, 0.5 H), 3.58 (d, J = 9.2 Hz, 1 H), 1.35 (br, 3H, CH_3); δ_C NMR (50 MHz, $CDCl_3$) mixture of rotamers 174.0 (s, CO_2Me), 156.7 and 156.0 (s, NCO), 143.4 (s, 2 C, Fmoc), 141.0 (s, 2 C, Fmoc), 127.5 (d, 2 C, Fmoc), 126.9 (d, 2 C, Fmoc), 124.7 (d, 2 C, Fmoc), 119.7 (d, 2 C, Fmoc), 69.3 and 68.4 (d, C-2), 67.7 (t, CH_2OC), 58.2 (d and t, 2 C, C-3 and C-6), 47.0 (d, $CHCH_2OC$), 41.0 (t, C-5), 16.4 (q, CH_3). ESI-MSMS m/z 368.22 (M^++1, 14), 351.12 (M^+–OH+1, 100).

(2*R*,3*S*,6*S*)-2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester (4). Compound **2** (1.29 g, 3.13 mmol) was dissolved in a mixture of THF (4.5 mL) and MeOH (2 mL). The mixture was cooled at 0 °C with an ice bath and 4.5 mL of 2.67 M LiOH were added in one portion. The reaction was stirred for 1.45 h at 0 °C and was quenched with 20% citric acid (approximately 3 mL) until pH 7 was reached. The reaction was basified with $NaHCO_3$ (526 mg, 6.26 mmol), diluted with THF (5.5 mL) and Fmoc-Cl (810 mg, 3.13 mmol) was added. The ice bath was removed after 30 min and the reaction was stirred for 16 h at room temperature. The mixture was then diluted with 5% Na_2CO_3 (90 mL), washed with Et_2O (4 × 35 mL), acidified at pH 2 with 37% HCl and extracted with CH_2Cl_2 (4 × 30 mL). The combined dichloromethane extracts were dried over Na_2SO_4 and concentrated under reduced pressure to yield pure **4** (1.064 g, 87%). (Found: C, 66.55; H, 5.89; N, 3.47. $C_{22}H_{23}NO_6$ requires C, 66.49; H, 5.83; N, 3.52%). $[\alpha]_D^{24}$ –1.6 (c 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 5 : 4 mixture of rotamers δ 7.74 (dd, J = 16.8, 7.6 Hz, 2 H, Fmoc CH), 7.59-7.49 (m, 2 H, Fmoc CH), 7.44 (dd, J = 14.4, 7.2 Hz, 2 H, Fmoc CH), 7.31 (dd, J = 14.4, 7.6 Hz, 2 H, Fmoc CH), 4.79 (t, J = 6.4 Hz, 0.55 H, H-6), 4.71 (t, J = 6.4 Hz, 0.45H, H-6), 4.60-4.40 (m, 2 H), 4.32-4.12 (m, 2.24 H), 4.52-3.93 (m, 1.76 H), 3.42 and 3.40 (2 s, 3 H, OCH_3), 3.09 (dd, J = 14.4, 8.0 Hz, 0.55 H, H-5) and 2.98 (dd, J = 14.4, 8.0 Hz, 0.45 H, H-5), 1.41 and 1.37 (2 d, J = 5.6 Hz, 3 H, CH_3); δ_C (50 MHz, $CDCl_3$) mixture of rotamers 174.9 and 174.7 (s, CO_2Me), 155.8 and 155.3 (s, NCO), 143.5 and 143.4 (s, 2 C, Fmoc), 141.1 (s, 2 C, Fmoc), 127.6 (d, 2 C, Fmoc), 127.0 (d, 2 C, Fmoc), 124.7 (d, 2 C, Fmoc), 119.8 (d, 2 C, Fmoc), 96.9 and 96.3 (d, C-6), 67.9 (t, CH_2OC), 64.7 and 64.3 (d, C-2), 61.2 and 60.7 (d, C-3), 55.2 (q, OCH_3), 47.0 (d, $CHCH_2OC$), 42.6 and 41.8 (t, C-5), 18.9 (q, CH_3). ESI-MSMS m/z 398.61 (M^++1, 18), 381.18 (M^+–OH+1, 100).

(2*R*,3*S*,6*R*)-2-Methyl-morpholine-3,4-dicarboxylic acid 4-(9*H*-fluoren-9-ylmethyl) ester (5). Ester **3** (1.10 g, 2.67 mmol) was treated according to the same procedure described for compound **4** to yield acid **5** (902 mg, 85%). (Found: C, 66.52; H, 5.86; N, 3.48. $C_{22}H_{23}NO_6$ requires C, 66.49; H, 5.83; N, 3.52%). $[\alpha]_D^{25}$ –64.7 (c 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) Mixture of rotamers 7.76 (d, J = 7.6 Hz, 2 H, Fmoc CH), 7.60 (m, 2 H, Fmoc CH), 7.39 (t, J = 7.6 Hz, 2 H, Fmoc CH), 7.30 (t, J = 7.6 Hz, 2H, Fmoc CH), 4.69 (t, J = 2.8 Hz, 1 H, H-6), 4.50-4.21 (m, 5 H), 3.89 (d, J = 13.2, 1 H), 3.60 (m, 1 H), 3.46 (s, 3 H, OCH_3), 1.49 (s, 3 H, CH_3); δ_C (50 MHz, $CDCl_3$) Mixture of rotamers 174.3 (s, CO_2Me), 156.6 (s, NCO), 143.6 and 143.5 (s, 2 C, Fmoc), 141.1 (s, 2 C, Fmoc), 127.6 (d, 2 C, Fmoc), 127.0 (d, 2 C, Fmoc), 125.0 (d, 2 C, Fmoc), 119.8 (d, 2 C, Fmoc), 96.6 (d, C-6), 69.0 (d, C-2), 68.2 (t, CH_2OC), 58.7 (d, C-3), 55.3 (q, OCH_3), 47.0 (d, $CHCH_2OC$), 44.2 (t), 20.4 (q, CH_3). ESI-MSMS m/z 398.64 (M^++1, 11), 381.15 (M^+-OH+1, 100).

General solution-phase peptide synthesis

General coupling procedure. Peptide couplings were performed in anhydrous dichloromethane using TBTU/DIPEA as the activating system and an equimolar amount of both coupling partners. After TLC indicated complete conversion, the solution mixture was concentrated under reduced pressure, dissolved in EtOAc and extracted with 1 M HCl, 5% Na_2CO_3, brine and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure and the obtained white solid was directly used for the deprotection step. Boc deprotections were carried out treating the Boc-peptide with a 1 : 1 mixture of CH_2Cl_2/TFA at 0 °C and were monitored by TLC. After complete conversion, the reaction mixture was concentrated under reduced pressure, and the resultant oil was co-evaporated with toluene and dried *in vacuo* to obtain the desired TFA salt as a white solid. Fmoc deprotections were carried out using a 30% mixture of diethylamine in CH_3CN.

Boc-D-Ala-D-Val-Mor-Gly-D-Leu-D-Val-OMe (I). Compound **10a** (130 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (3 mL) and DIPEA (128 µL, 0.75 mmol) was added. To the resultant solution, Boc-D-Ala-OH (47 mg, 0.25 mmol) and TBTU (80 mg, 0.25 mmol) were sequentially added. The reaction mixture was stirred for 16 h at room temperature and then CH_2Cl_2 was removed under reduced pressure. The resultant oil was dissolved in EtOAc, washed with 1 M HCl, 5% Na_2CO_3 and brine, and dried over anhydrous Na_2SO_4. The solution was filtered and the residue was eluted over silica gel (Et_2O–MeOH 60 : 1 to 30 : 1) to yield peptide **I** as a white solid (160 mg, 91%). (Found: C, 56.81; H, 8.42; N, 11.97. $C_{33}H_{58}N_6O_{10}$ requires C, 56.72; H, 8.37; N, 12.03%). ESI-MSMS m/z 721.59 (M^++Na, 4), 621.43 (M^+–Boc+Na, 100). See the ESI for NMR data.†

Boc-D-Ala-D-Val-[(6*S*)-methoxy]-Mor-Gly-D-Leu-D-Val-OMe (II). Compound **10b** (93 mg, 0.17 mmol) was dissolved in CH_2Cl_2 (3 mL) and DIPEA (88 μL, 0.51 mmol) was added. To the resultant solution, Boc-D-Ala-OH (32 mg, 0.17 mmol) and TBTU (55 mg, 0.17 mmol) were sequentially added. The reaction mixture was stirred 16 h at room temperature and then CH_2Cl_2 was removed under reduced pressure. The resultant oil was dissolved in EtOAc, washed with 1 M HCl, 5% Na_2CO_3 and brine, and dried over anhydrous Na_2SO_4. The solution was filtered and the residue was eluted over silica gel (Et_2O–MeOH 60 : 1 to 30 : 1) to yield peptide **II** as a white solid (117 mg, 94%). (Found: C, 56.11; H, 8.41; N, 11.37. $C_{34}H_{60}N_6O_{11}$ requires C, 56.03; H, 8.30; N, 11.53%). ESI-MSMS *m/z* 751.59 (M^++Na, 20), 651.45 (M^+–Boc+Na, 100). See the ESI for NMR data.†

Boc-D-Ala-D-Val-[(6*R*)-methoxy]-Mor-Gly-D-Leu-D-Val-OMe (III). Compound **10c** (89 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (3 mL) and DIPEA (82 μL, 0.48 mmol) was added. To the resultant solution, Boc-D-Ala-OH (30 mg, 0.16 mmol) and TBTU (51 mg, 0.16 mmol) were sequentially added. The reaction mixture was stirred for 16 h at room temperature and then CH_2Cl_2 was removed under reduced pressure. The resultant oil was dissolved in EtOAc, washed with 1 M HCl, 5% Na_2CO_3 and brine, and dried over anhydrous Na_2SO_4. The solution was filtered and the residue was eluted over silica gel (Et_2O–MeOH 60 : 1 to 30 : 1) to yield peptide **III** as a white solid (109 mg, 93%). (Found: C, 56.10; H, 8.36; N, 11.42. $C_{34}H_{60}N_6O_{11}$ requires C, 56.03; H, 8.30; N, 11.53%). ESI-MSMS *m/z* 751.58 (M^++Na, 10), 651.38 (M^+–Boc+Na, 100). See the ESI for NMR data.†

Fmoc-D-Val-Dox-Gly-D-Leu-D-Val-OMe (11). 2,6-Lutidine (215 μL, 1.85 mmol) was added to a mixture of peptides **9b** and **9c** (300 mg, 0.61 mmol) in CH_2Cl_2 (5 mL). Solid Fmoc-D-Val-Cl (218 mg, 0.61 mmol) was added in small portions. The reaction mixture was stirred at room temperature for 4 h and dichloromethane was then removed under reduced pressure. The resultant oil was dissolved in EtOAc, washed with HCl 1 M, 5% Na_2CO_3 and brine, and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated to dryness to yield a solid, which was dissolved in toluene (15 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (5 mg, 0.026 mmol). The reaction mixture was placed in a single-necked round-bottomed flask equipped with a reflux condenser and dropping funnel containing approximately 4 g of 4 Å molecular sieves. The mixture was refluxed for 3 h. Then it was cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (Et_2O–MeOH 60 : 1) to yield compound **11** as a white solid (290 mg, 63%). (Found: C, 64.56; H, 7.25; N, 9.27. $C_{40}H_{53}N_5O_9$ requires C, 64.24; H, 7.14; N, 9.36%). $[\alpha]_D^{20} = -1.0$ (*c* 0.75, CH_3CN); δ_H (400 MHz, $CDCl_3$) 7.69 (d, $J = 7.5$ Hz, 2 H, Fmoc CH), 7.49 (t, $J = 7.4$ Hz, 2 H, Fmoc CH), 7.33 (t, $J = 7.4$ Hz, 2 H, Fmoc CH), 7.24 (td, $J = 7.4$, 1.4 Hz, 2 H, Fmoc CH), 7.19 (m, 1 H, Gly NH), 6.47 (m, 2 H, D-Val_2 NH + D-Leu NH), 6.30 (s, 1 H, H-6), 5.89 (d, $J = 4.4$ Hz, 1 H, H-5), 5.70 (d, $J = 5.2$ Hz, Fmoc NH), 5.02 (q, $J = 6.0$ Hz, 1 H, H-2), 4.87 (s, 1 H, H-3), 4.36 (m, 2 H, D-Val_2 H-α + D-Leu H-α), 4.30 (m, 3 H, D-Val_1 H-α + fluorenyl-CH_2-CH), 4.13 (t, $J = 7.0$ Hz, 1 H, fluorenyl-CH_2–C*H*), 3.84 (dd, $J = 16.6$, 5.6, 1 H, Gly H-α), 3.62 and 3.59 (s, 3 H, OMe), 3.57 (m, 1 H, Gly H-α), 2.06 (m, 2 H, D-Val_1 H-β + D-Val_2 H-β), 1.58-1.36 (m, 3 H, D-Leu H-β + D-Leu H-γ), 1.19 (d, $J = 6.6$ Hz, 3 H, CH_3–C-2), 1.01 (d, $J = 6.2$ Hz, 3 H, D-Leu H-δ), 0.95 (d, $J = 6.2$ Hz, 3 H, D-Leu H-δ), 0.83 (d, $J = 6.2$ Hz, 3 H, D-Val H-γ), 0.79 (m, 9 H, D-Val H-γ). δ_C (50 MHz, $CDCl_3$) 171.9 (s, CO), 171.6 (s, CO), 170.0 (s, CO), 168.6 (s, CO), 168.0 (s, *C*O_2Me), 157.2 (s, NCO), 143.5 (s, Fmoc), 141.1 (s, Fmoc), 129.2 (d, Fmoc) 127.7 (d, Fmoc), 127.1 (d, Fmoc), 125.0 (d, Fmoc), 124.9 (d, Fmoc), 119.9 (d, C-6), 103.0 (d, C-5), 70.1 (d), 67.6 (t, *C*H_2OC), 57.4 (d), 56.9 (d), 56.6 (d), 52.0 (d), 47.1 (d, *C*HCH$_2$OC), 43.5 (t), 40.9 (t), 31.2 (d), 30.4 (d), 24.8 (d), 23.1 (q, CH_3), 22.1 (q, CH_3), 19.4 (q, CH_3), 19.0 (q, CH_3), 18.8 (q, CH_3), 18.0 (q, CH_3), 17.3 (q, CH_3). ESI-MSMS *m/z* 770.49 (M^++Na, 6), 548.34 (M^+–Fmoc+Na, 100).

Boc-D-Ala-D-Val-Dox-Gly-D-Leu-D-Val-OMe (IV). Compound **11** (120 mg, 0.16 mmol) was treated with 30% Et_2NH in CH_3CN (3 mL). The Fmoc deprotection was monitored by TLC. When complete conversion was obtained, volatiles were removed under reduced pressure and the residue was eluted over silica gel (Et_2O–MeOH 30 : 1 to pure MeOH). The isolated solid was dissolved in 4 mL of dichloromethane and DIPEA (82 μL, 0.48 mmol) was added. To the resultant solution, Boc-D-Ala-OH (30 mg, 0.16 mmol) and TBTU (51 mg, 0.16 mmol) were sequentially added. The reaction mixture was stirred for 16 h at room temperature and then CH_2Cl_2 was removed under reduced pressure. The resultant oil was dissolved in EtOAc, washed with 1 M HCl, 5% Na_2CO_3, and brine, and dried over anhydrous Na_2SO_4. The solution was filtered and the residue was purified by flash chromatography on silica (Et_2O–MeOH 60 : 1 to 30 : 1) to yield peptide **IV** as a white solid (96 mg, 86%). (Found: C, 56.92; H, 8.14; N, 12.00. $C_{33}H_{56}N_6O_{10}$ requires C, 56.88; H, 8.10; N, 12.06%). ESI-MSMS *m/z* 719.6 (M^++Na, 14), 619.5 (M^+–Boc+Na, 100). See the ESI for NMR data.†

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