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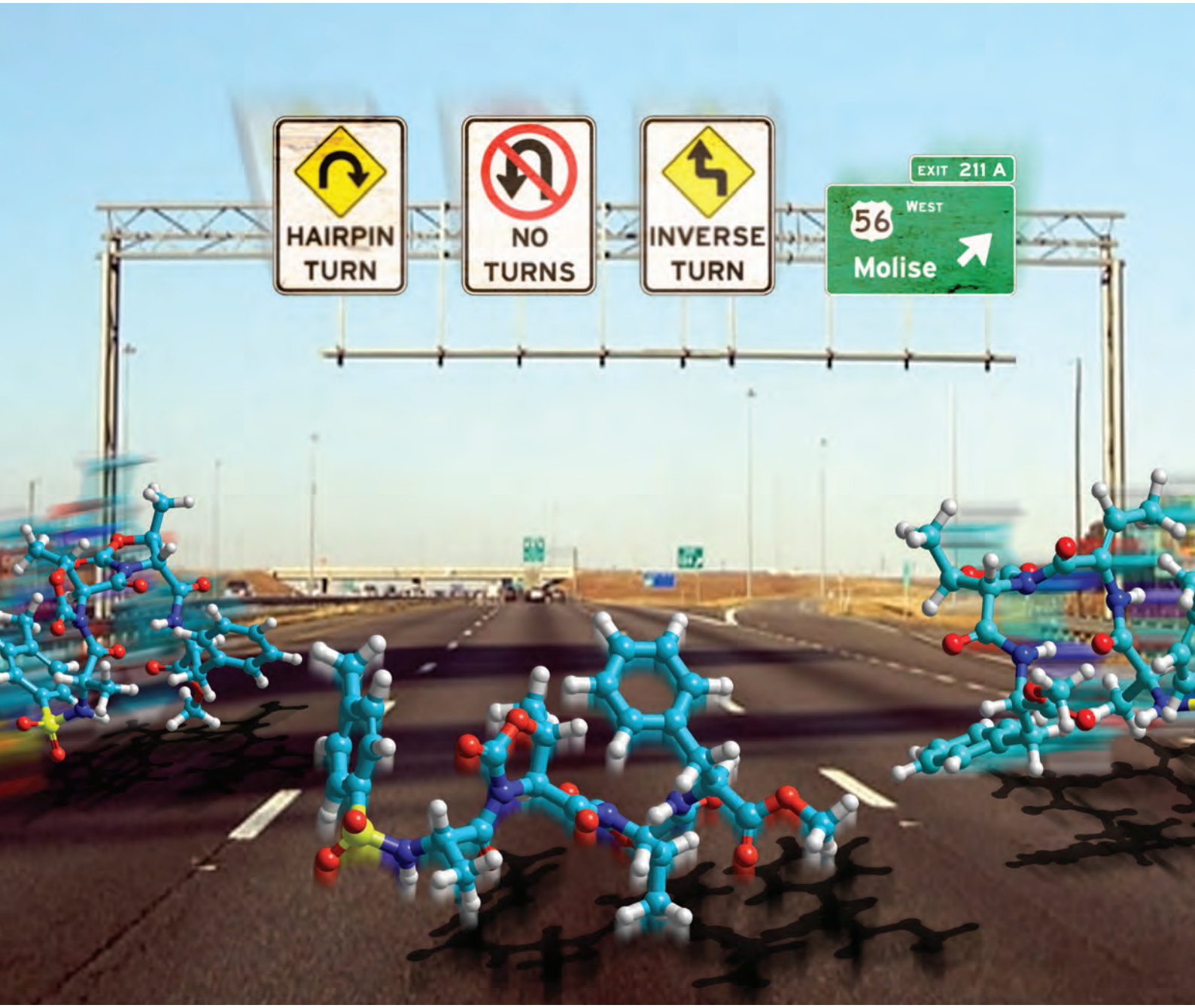

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In-peptide synthesis of di-oxazolidinone and dehydroamino acid–oxazolidinone motifs as β-turn inducers†

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Small and easy-to-do mimetics of β-turns are of great interest to interfere with protein–protein recognition events mediated by β-turn recognition motifs. We propose a straightforward procedure for constraining the conformation of tetrapeptides lacking a pre-formed scaffold. According to the stereochemistry array, *N*-Ts tetrapeptides including Thr or PhSer (phenylserine) at the positions 2 or 3 gave rise in a single step to the sequences Oxd[2]-Oxd[3] or ΔAbu[2]-Oxd[3] (Oxd, oxazolidin-2-one; ΔAbu, 2,3-dehydro-2-aminobutyric). These pseudo-Pro residues displayed highly constrained ϕ, ψ, and χ dihedral angles, and induced clear β-turns or inverse turns of type I or II, as determined by extensive spectroscopic and computational analyses.

Introduction

Interactions between proteins (PPI) are important for the majority of biological functions and cellular signaling.[1] Systematic studies have revealed that there is typically a small cluster of residues at the interface of the proteins that contributes the majority of the recognition or binding affinity. Very often, these key regions adopt well defined turn structures.[2] As a consequence, small molecules mimicking the 3D features of the turn regions can find applications as therapeutic agents.

The β-turn occurs where the polypeptide backbone reverses direction, and consists of four amino acid residues *i* to *i* + 3 in which the distance between Cα*i* and Cα*i* + 3 is about 7 Å; according to the dihedral angles ϕ and ψ of the residues *i* + 1 and *i* + 2, the turns are classified in different types.

Small mimetics of β-turns, such as the privileged structures benzodiazepines, Freidinger lactams, internal or external bicyclic dipeptide mimetics, spirocyclic dipeptides, *etc.*, have been extensively investigated and utilized to discover compounds that can mimic or disrupt β-turn-mediated recognition events.[3]

However, the preparation of the scaffolds and their incorporation in the sequence may require multi-step procedures, resulting in low overall yields. Besides, most synthetic methods lack flexibility and are therefore not suited to introduce diversity.[3] As a consequence, the development of a high yielding, single step procedure for the introduction of a conformational constraint that forces the peptide to adopt a β-turn is currently of considerable interest.

In this work we report a straightforward procedure for constraining the conformation of tetrapeptides lacking a preformed scaffold. The resulting β-turn mimetics include 2-oxo-1,3-oxazolidine-4-carboxylate rings (or oxazolidin-2-one, in short: Oxd), that we recently proposed as constrained pseudo-Pro residues.[4]

Pro strongly impacts the structural and conformational properties of peptide and protein backbones and their molecular recognition.[5] It does not have a hydrogen on the amide group and therefore cannot act as a H-bond donor. The cyclic structure forces the ϕ angle to about −65°, therefore Pro is known as a classical breaker of both the α-helical and β-sheet structures in proteins and peptides, while it promotes the formation of β-turns.[2,6]

Besides, due to the small free enthalpy difference between the *cis* and *trans* Xaa-Pro bond isomers of 2.0 kJ mol^{-1} (compared to 10.0 kJ mol^{-1} for other peptide bonds), there is a relatively high intrinsic probability of 30% *cis* conformation at r.t.[7] Interestingly, the *cis–trans* interconversion of X-Pro is one of the rate-determining steps in protein folding.[8]

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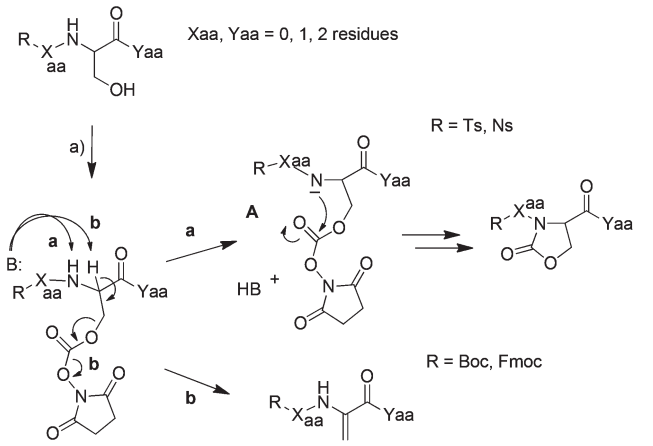
†Electronic supplementary information (ESI) available: ^{1}H-NMR of **2a**, **c**, **d**, and **3b**, **c** in 8 : 2 DMSO-d_6–H_2O; selected ^{1}H-NMR chemical shifts of **2a**, **c**, **d**, and **3b**, **c** in different solvents; amide NH FT-IR of **2d**, and **3b**; NH chemical shift of **2a** and **3c** in $CDCl_3$–DMSO-d_6 0–8%; ROESY cross-peaks for **2a**, **c**, **d**, and **3b**, **c**; Structure of the intermediate anion **A**; ϕ and ψ angles of the residues *i* + 1, *i* + 2 for **2a**, **c**, **d**, and **3b**, **c**; ECD spectra of **2a**, **2d**, and **3b** in DCM and MeOH; reproductions of the ^{1}H- and ^{13}C-NMR of compounds **2** and **3**. See DOI: 10.1039/c3ob40357b

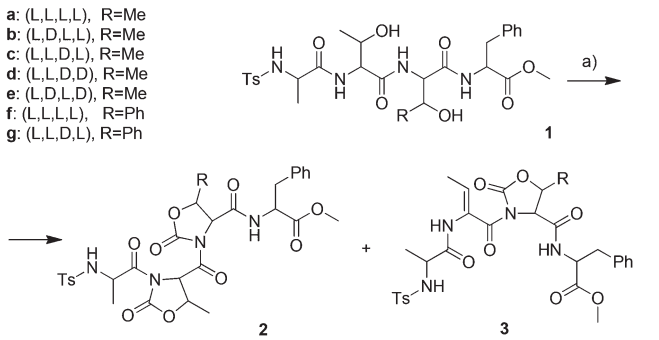
In order to understand the relationship between peptide bond geometry and bioactivity, many synthetic Pro analogues have been developed that provide restrictions of the Xaa-Pro conformation. In general, the modifications consist in ring substitutions with alkyl and aromatic groups, incorporation of heteroatoms, or the expansion or contraction of the five-membered ring.[9]

To take advantage of the conformational bias exerted by the Oxd pseudo-Pro, we planned to synthesize diastereomeric *N*-sulfonyl tetrapeptides containing two consecutive Oxd rings, as β-turn inducers. According to the stereochemistry array of the amino acids, the reaction of two β-hydroxy α-amino acids (Thr or *threo*-phenylserine, PhSer) already present within the sequences at the positions 2 and 3, with bis(succinimidyl)carbonate (DSC), afforded two consecutive Oxd or a 2,3-dehydro-2-aminobutyric acid (ΔAbu) in 2 and a Oxd in 3. These combinations resulted in highly constrained extended or folded structures, in particular turns or inverse turns of type I and II, and are especially interesting because they can control not only the ϕ and ψ dihedral angles of the backbone but also the χ angles of the side chains.

Peptides containing Oxd rings constitute an infrequent but remarkable class of peptidomimetics. They have found some applications in medicinal chemistry,[10] in the construction of foldamers[11] or as self-assembling scaffolds forming nanostructures.[12] Also the compounds with the ΔAbu-Oxd sequence are of some interest, since they contain two distinct secondary structure-forming elements. The dehydroamino acids are well known β-turn inducers; for instance, sequential placement of dehydroPhe (ΔPhe) in oligomers gave repeated β-turns forming 3_{10} helices.[13]



Scheme 1 Different reactivity of the *N*-carbamate and *N*-sulfonyl oligopeptides. (a) DSC, cat. base (B:).



Scheme 2 Reactions of the sulfonyl-peptides **1a–e**, containing L- or D-Thr at positions 2 and 3, and of **1f**, **g** containing L-Thr at 2 and *threo* L- or D-PhSer at 3, respectively. (a) DSC, cat. DIPEA.

Results and discussion

Synthesis of tetrapeptides containing di-Oxd and ΔAbu-Oxd

Very recently, we reported the synthesis of Oxd rings directly within peptide sequences, by treatment of *N*-arylsulfonyl peptides containing L- or D-configured Ser with DSC and a catalytic amount of a base, in solution or in the solid phase (Scheme 1, path a).[4]

Apparently, the reaction was due to the presence of the *N*-sulfonyl group; indeed, the reaction of the corresponding Fmoc- or Boc-peptides under the same conditions with carbonates or dicarbonates gave elimination to dehydroalanine (ΔAla), as reported in the literature (Scheme 1, path b).[13]

Interestingly, peptides having the sulfonyl group directly connected to the Ser, or separated by one or two amino acids, gave similar results (Scheme 1, Xaa = 0, 1, or 2 residues).[4] This observation prompted us to perform in a single step the simultaneous cyclization of sulfonyl-oligopeptides containing two consecutive ring-forming residues, to afford highly rigid structures.

Accordingly, we prepared a series of diastereomeric tetrapeptides containing two Thr residues, or Thr and *threo*-PhSer, at the positions 2 and 3, and we reacted the tetrapeptides (Scheme 2) with a moderate excess of DSC and a catalytic amount of diisopropylethylamine (DIPEA).

The preparation of the *N*-tosyl tetrapeptides **1a–g** was conducted by coupling the amino acids under MW irradiation, using a microwave oven specifically designed for organic synthesis,[14] with HBTU/HOBt as activating agents (Experimental section, Table 3). *N*-Tosyl (Ts)-Ala was prepared as described in the literature;[15] L- and D-*threo*-PhSer were obtained *via* optical resolution of the racemate,[16] since the resolution by enzymatic hydrolysis[17] gave poor results.

The reaction of Ts-Ala-Thr-Thr-PheOMe (**1a**) with 2.2 equiv. of DSC in 3 : 1 DCM–DMF and in the presence of 0.1 equiv. of DIPEA (Scheme 2, entry 1) provided the corresponding di-Oxd-containing Ts-Ala-Oxd-Oxd-PheOMe (**2a**) in excellent yield after isolation by flash chromatography (Table 1).

On the other hand, the reaction of Ts-Ala-D-Thr-Thr-PheOMe (**1b**), under the same reaction conditions, gave exclusively a tetrapeptide containing ΔAbu at position 2, Ts-Ala-ΔAbu-Oxd-PheOMe (**3b**), while the tetrapeptide containing two Oxd (**2b**) was observed only in trace amounts (Scheme 2, and Table 1, entry 2).

Table 1 Synthesis of the constrained peptidomimetics **2** or **3** from the stereoisomers **1**

Entry	1	Ala[1]	Thr[2]	Xaa[3],	R	Phe[4]	Solvent[a]	2[b] (%)	3[b] (%)
1	**a**	L	L	L	Me	L	DCM–DMF	87	tr.
2	**b**	L	D	L	Me	L	DCM–DMF	—	90
3	**c**	L	L	D	Me	L	DCM–DMF	67	23
4	**c**	”	”	”	Me	”	DCM	85	9
5	**c**	”	”	”	Me	”	DMF	5	78
6	**d**	L	L	D	Me	D	DCM–DMF	90	—
7	**e**	L	D	L	Me	D	DCM and/or DMF	tr.[c]	tr.[c]
8	**f**	L	L	L	Ph	L	DCM–DMF	92	—
9	**g**	L	L	D	Ph	L	DCM–DMF	93	tr.

[a] All compounds **1a–g** were tested in different solvent mixtures (3 : 1 DCM–DMF, DCM, DMF); with the exception of entries 3–5, the reaction outcomes were practically unaffected, therefore these results are not shown. [b] Determined after purification by flash chromatography over silica gel; tr. = traces. [c] The rest being the reagent.

The reaction of Ts-Ala-Thr-D-Thr-PheOMe (**1c**) under the same conditions gave a 74 : 26 mixture of Ts-Ala-Oxd-D-Oxd-PheOMe (**2c**) and Ts-Ala-ΔAbu-D-Oxd-PheOMe (**3c**) (entry 3). The reaction outcome changed upon varying the solvent utilized; in pure DCM the yield of **2c** increased to 85% (entry 4), while in DMF the reaction afforded mainly **3c**, with a 78% yield (entry 5).

The tetrapeptide Ts-Ala-Thr-D-Thr-D-PheOMe (**1d**) exclusively gave Ts-Ala-Oxd-D-Oxd-D-PheOMe (**2d**) (entry 6), while Ts-Ala-D-Thr-Thr-D-PheOMe (**1e**) failed to give either **2** or **3** in significant amounts (entry 7) under a variety of reaction conditions (not shown).

Finally, the reaction was repeated with the sequences Ts-Ala-Thr-PhSer-PheOMe (**1f**) or Ts-Ala-Thr-D-PhSer-PheOMe (**1g**), which gave the di-Oxd compounds **2f** and **2g** in very satisfactory yields (entries 8 and 9). These peptides include a 2-oxo-5-phenyloxazolidine-4-carboxylate residue at position 3, which might effectively represent a constrained Phe mimetic, with fixed ϕ, ψ and χ dihedral angles (Table S7†).

This result suggests the opportunity to develop analogues of proteinogenic or unusual amino acids, carrying the side chain at the position 5 of the Oxd ring. Indeed, the literature reports many procedures for the stereocontrolled preparation of a variety of β-hydroxy α-amino acids.[18] Nevertheless, the synthesis of Oxds functionalized with different side chains is beyond the scope of this work, which primarily addresses the synthesis of model tetrapeptides and investigation of the secondary structures.

Epimerization during the reactions was excluded on the basis of the comparison of the NMR and HPLC analyses of the compounds, including the HPLC analysis on a chiral stationary phase (see General methods).

From the comparison of the results (entries 1–9) it appeared that the stereochemistry array exerted a major bias in determining the cyclization *versus* the elimination at position 2. Homochiral sequences at positions 1 and 2 tended to give exclusively or predominantly the di-Oxd compounds, as observed for **1a**, **1c**, and **1d**. On the contrary, the peptide **1e** containing the sequence L-Ala-D-Thr failed to give **2** or **3** in significant amounts, and **1b** solely afforded the ΔAbu-Oxd sequence. The diastereomeric peptides **1f**, **1g** containing PhSer behaved in a similar way to the corresponding peptides **1a** and **1c** (the formation of **3g** was observed only in trace amounts in different solvents).

The proposed reaction mechanism of Scheme 1, path **a**, postulates that for n = 1 or 2, as occurring for the compounds **1a–g**, the electron-poor aromatic ring of the arylsulfonylamido group might effectively stabilize the anionic intermediate **A** by π-stacking interaction (ESI†), thus driving formation of the Oxd ring. Possibly, the diastereoisomer **1b** adopted in solution a conformation which allowed promoting the cyclization at position 3, but not at 2, therefore giving elimination, as expected on the basis of the literature. For **1c**, the different results observed for the entries 3 to 5 could be attributed to a preference for different conformations in the different solvents. As for **1e**, showing alternating absolute stereochemistry, it appeared that the compound was unable to achieve cyclization either at 2 or 3; the compound did not eliminate to a significant extent either, as observed by treatment with different bases in different solvents at r.t. or under heating.

The mild cleavage of the arylsulfonyl group[19] was discussed previously.[4,20] The tosyl group was removed in good yield with iodotrimethylsilane,[21] while the treatment with SmI_2–pyrrolidine–water[22] was less efficient.

Conformational analyses of the di-Oxd and ΔAbu-Oxd peptides

To investigate the conformational bias exerted by the two consecutive L- or D-configured Oxd, or ΔAbu and L- or D-Oxd residues at positions 2 and 3, on the overall structure of the peptides, we analyzed the model compounds **2a**, **2c**, **2d**, and **3b**, **3c**, by FT-IR and NMR spectroscopy.

FT-IR spectroscopy was utilized to analyze the amide N–H stretching regions; generally, non H-bonded amide protons show a peak above 3400 cm^{-1}, while H-bonded amide NH bonds exhibit a peak below 3400 cm^{-1}.[23]

The FT-IR absorption spectrum of **2d** only showed a strong band above 3400 cm^{-1} (ESI, Fig. S1†). The spectra of **2a**, **2c**, **3b**, and **3c** (ESI, Fig. S1†) showed a major peak at about 3400 cm^{-1}, and a second peak at about 3340 cm^{-1}. The latter became predominant in **3c**, suggestive of a significant population of H-bonded conformations in equilibrium with non-

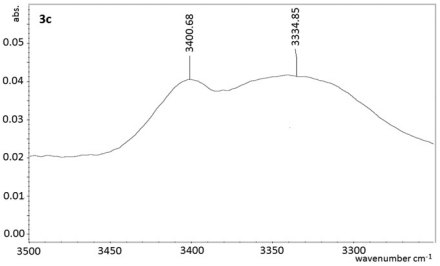


Fig. 1 Amide NH stretching regions of the IR absorption spectra for samples of tetrapeptide **3c** (3 mM in DCM, r.t.).

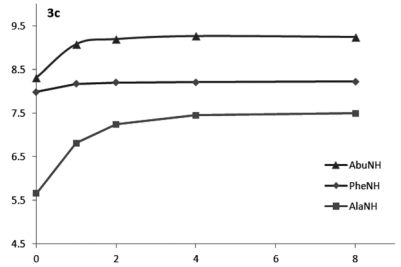


Fig. 2 Variation of NH proton chemical shift (ppm) of **3c** as a function of increasing percentages of DMSO-d_6 to the $CDCl_3$ solution (v/v).

bonded structures. The spectrum of **3c** dissolved in DCM is reported in Fig. 1.

The ^{1}H-NMR experiments were done at 400 MHz in $CDCl_3$ or in 8 : 2 DMSO-d_6–H_2O. 2D gCOSY experiments were utilized for the unambiguous assignment of the resonances. In each solvent, all spectra showed a single set of sharp resonances, consistent with conformational homogeneity or a fast equilibrium between slightly different conformers. For most compounds, the ^{1}H-NMR resonances showed modest variations of the chemical shifts in the two solvents (Table S1†) suggesting that the peptidomimetic sequences were conformationally stable; exceptions are highlighted in Table S1.†

It has been shown that the Oxd confers on the preceding amide bond an exclusive *trans* conformation.[11] The ^{1}H-NMR analyses of all of the compounds showed a significantly downfield position of the Hα proton of the residues preceding the Oxd rings (ESI, Table S1†). For instance, the resonances of AlaHα in Ts-Ala-Thr-Thr-PheOMe (**1a**) and Ts-Ala-ΔAbu-L-(5′-Me-Oxd)-PheOMe (**3b**) appeared at about 3.9 and 3.8 ppm ($CDCl_3$), respectively, while in Ts-Ala-(5′-Me-Oxd^2)-(5′-Me-Oxd^3)-PheOMe (**2a**) AlaHα was at about 5.2 ppm. On the other hand, in the same compound **2a**, Oxd^{3}H4 was at 4.2 ppm, while Oxd^{2}H4 was at about 5.3 ppm, for the deshielding effect of Oxd^{3}C=O. These observations were compatible with a non-conventional, CH···O=C intramolecular H-bond,[24] confirming the *trans* conformation of the amide bond between the Oxd and the preceding, deshielded residue.

The occurrence of intramolecular H-bonds in **2a**, **2c**, **2d**, **3b**, and **3c**, was evaluated by analyzing the variation of the NH proton chemical shifts upon addition of increasing percentages of DMSO-d_6 to 2 mM solutions of the compounds in $CDCl_3$. As representative example, the titration curves of **3c** are shown in Fig. 2. The titration of **2a** gave no evidence of H-bonds involving PheNH nor AlaNH, since these signals exhibited a considerable downfield shift;[25] **2c**, **2d** gave similar results (ESI†). On the contrary, the titration of **3c** (Fig. 2) and **3b** (ESI†) revealed that PheNH, but not AlaNH nor ΔAbuNH, was much less sensitive, therefore less accessible, accounting for a H-bonded structure.

Variable temperature (VT)-^{1}H-NMR experiments in $CDCl_3$ and 8 : 2 DMSO-d_6–H_2O were also utilized to deduce the presence of intramolecular H-bonds (Table 2). For almost all compounds, the comparatively lower VT-^{1}H-NMR $\Delta\delta/\Delta t$ parameters

Table 2 $\Delta\delta/\Delta t$ values (ppb K^{-1}) for the amide protons of **2a**, **2c**, **2d**, and **3b**, **3c**, in $CDCl_3$ and 8 : 2 DMSO-d_6–H_2O; solvents: S1 = $CDCl_3$; S2 = 8 : 2 DMSO-d_6–H_2O; amino acid stereochemistry has been omitted

Compd	Solvent	Ala^{1}NH	ΔAbu^{2}NH	Phe^{4}NH
2a	S1	−2.7	—	−2.4
	S2	−6.1	—	−3.9
3b	S1	−13.5	−8.0	−3.8
	S2	−5.0	−7.2	−4.1
2c	S1	−2.3	—	−1.2
	S2	−6.3	—	−3.8
3c	S1	−9.0	−4.4	−4.0
	S2	−6.5	−7.0	−4.1
2d	S1	−1.7	—	−2.7
	S2	−6.0	—	−4.2

of PheNH with respect to AlaNH (and ΔAbuNH) were suggestive of a moderate preference for conformations having PheNH involved in a H-bond ($|\Delta\delta/\Delta t|$ < or close to 2.5 ppb K^{-1}).[23,26] The only exception was observed for **2d** in $CDCl_3$, since the $\Delta\delta/\Delta t$ (ppb K^{-1}) of AlaNH was lower than that of PheNH (−1.7 and −2.7, respectively).

From the comparison of the results above discussed for the Oxd–Oxd compounds (**2**), it appeared that the possible existence or not of H-bonds was differently supported by the methods employed. Reasonably, the inconsistencies reflected the existence of equilibria between H-bonded and non-H-bonded structures. As for the ΔAbu-Oxd compounds (**3**), all of the evidence was highly coherent in support of a H-bonded PheNH.

To determine the preferred conformations in solution and their dynamic behaviours, the compounds were analyzed by 2D-ROESY in 8 : 2 DMSO-d_6–H_2O and molecular dynamics (MD) simulations. DMSO-d_6 or DMSO-d_6–H_2O mixtures have been recommended by several authors as biomimetic media.[27] Structures consistent with the spectroscopic analyses were obtained by MD using the distances derived from ROESY as constraints. The intensities of the ROESY cross-peaks were ranked to infer the distances (Tables S2–6, ESI†). The ω bonds were set at 180°, since the absence of Hα*i*-Hα(i + 1) cross-peaks excluded *cis* peptide bonds. Simulations were conducted in a box of explicit water molecules. Random structures were generated by unrestrained high-temperature MD; the structures were subjected to high-temperature restrained MD with a scaled force field, followed by a simulation with full restraints.

Finally, the system was gradually cooled, and the structures were minimized with the AMBER force field.[28] The results were clustered by the RMSD analysis of the backbone atoms.

For Ts-Ala-(5′-Me-Oxd)-(5′-Me-Oxd)-PheOMe (**2a**), the analysis gave two clusters comprising altogether more than 90% of the structures. For each cluster, the representative geometries A and B with the lowest internal energy were selected and analyzed (Fig. 3). The occurrence of these two different structures, which differ almost exclusively by the opposite orientation of the Phe residue, reflected the observation of contradictory cross-peaks: the cross-peaks PheNH-Oxd^{3}H4 and PheNH-Oxd^{3}H5 accounted for the conformation A, while the cross peak PheNH-Oxd^{2}H5 was coherent with B. Possibly, the two structures represented conformers in fast equilibrium.

To investigate the dynamic behaviour of **2a** (A) and (B), unrestrained MD were performed for 10 ns in a box of standard TIP3P water molecules. The simulations showed the conversion of one conformation into the other. The analyses of the trajectories of the conformer (A) revealed very few structures compatible with a γ-turn centered on Oxd^3 with an explicit H-bond between PheNH and Oxd^{2}C═O, while (B) gave no evidence of secondary structures.

For Ts-Ala-(5′-Me-Oxd)-D-(5′-Me-Oxd)-PheOMe (**2c**) and Ts-Ala-(5′-Me-Oxd)-(5′-Me-Oxd)-D-PheOMe (**2d**), the analyses gave one major cluster each comprising about 80% of the structures. For each compound's cluster, the representative geometries with the lowest internal energy are shown in Fig. 3. These structures **2c** (C) and **2d** are compatible with a well-defined type II β-turn secondary structure (Table S7†). The structures **2c** (C) and **2d** were analyzed by unrestrained MD; besides the different random conformations, the analysis of the trajectories of **2c** (C), but not of **2d**, revealed an explicit H-bond between PheNH and the AlaC═O, as shown in **2c** (D), Fig. 3.

The conformational analyses of the peptides **3b**, **3c** containing in their sequences ΔAbu^2 and L- or D-Oxd^3, respectively, gave one cluster each, comprising the large majority of the structures; the representative geometries are shown in Fig. 4.

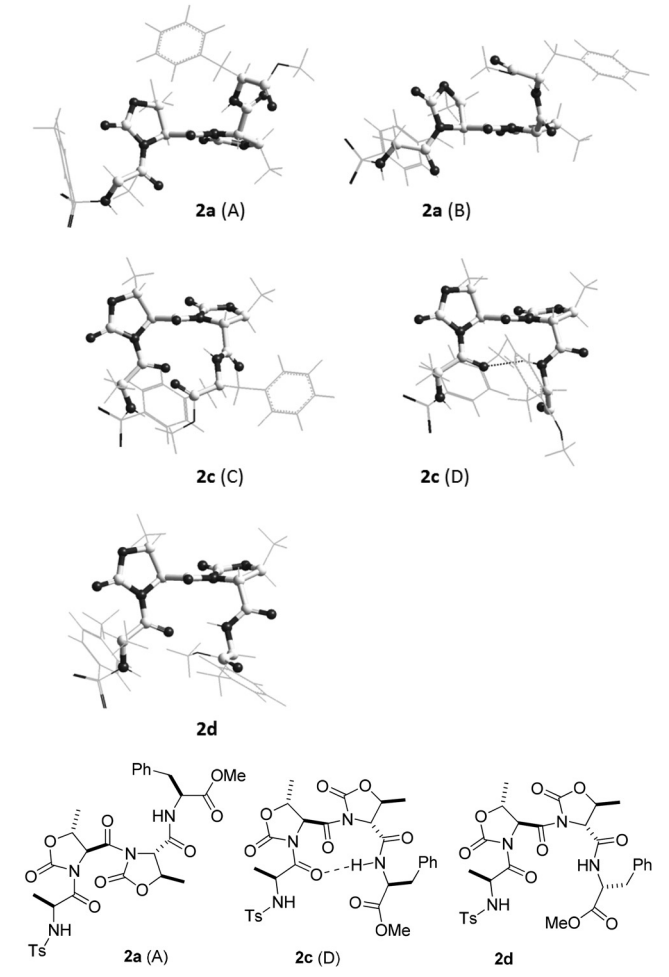


Fig. 3 Top. Representative low-energy structures of **2a** (A and B), **2c** (C), and **2d** consistent with ROESY analysis, calculated by restrained MD; low energy structure of **2c** (D) calculated by unrestrained MD. All structures determined in a 30 × 30 × 30 Å box of standard TIP3P water molecules. Backbones and Oxd are rendered in balls and cylinders, the rest in sticks. The peptide bonds connecting Oxd^2-Oxd^3 are shown horizontally and perpendicularly with respect to the viewer. Bottom. Sketches of the extended structure of **2a** (A), and of the folded **2c** (D) and **2d**.

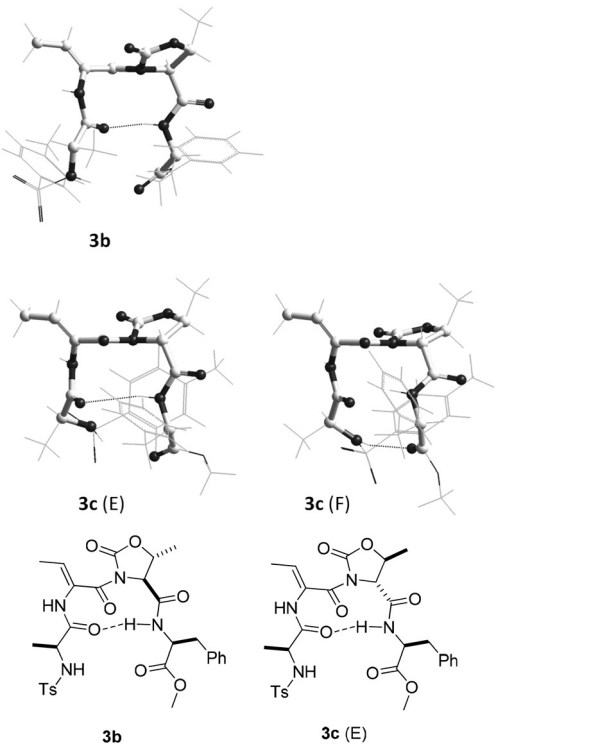


Fig. 4 Top. Representative low-energy structures of **3b** and **3c** (E) consistent with ROESY analysis, calculated by restrained MD; low energy structure of **3c** (F) calculated by unrestrained MD. All structures determined in a 30 × 30 × 30 Å box of standard TIP3P water molecules. Backbones and Oxd are rendered in balls and cylinders, the rest in sticks. The peptide bonds connecting ΔAbu^2-Oxd^3 are shown horizontally and perpendicularly with respect to the viewer. Bottom. Sketches of the folded structures **3b** and **3c** (E).

The calculated geometry of ΔAbu closely matched that of dehydroamino acids reported in the literature.[13,29]

The structure **3b**, stabilized by an explicit H-bond between AlaC═O and PheNH, was compatible with a type I β-turn. The structure of **3c** (E) nicely reproduced an inverse type I β-turn (Table S7†), and showed a H-bond between AlaC═O and PheNH. The unrestrained MD simulations also revealed a tendency for structures characterized by a H-bond between PheC═O and AlaNH, such as the representative **3c** (F), Fig. 4.

To further confirm the presence of folded structures, we used Electronic Circular Dichroism (CD) spectroscopy. Spectra of model peptides **2a**, **2d**, and **3b**, were recorded both in dichloromethane and in methanol.

The spectra in dichloromethane (Fig. 5) showed a negative band centered at about 230 nm, less intense for **2a**. These spectral features could be indicative of a significant population of bent conformers, in particular in the case of **2d** and **3b**. In fact, a negative nπ* ECD band near 225 nm is generally observed in the presence of β-turn structures (I or II type).[23,30] The observed different intensities of ECD signals were in agreement with ROESY and MD data which evidenced homogeneous conformations for **2d** and **3b**.

By moving from dichloromethane to methanol, intensities of the negative ECD bands slightly reduced for all compounds (see ESI, Fig. S3†). This spectral behavior could be ascribed to a dependence of the conformer population on the polarity and/or the competitive nature of the solvent.

Taken together, all of the experimental evidence supports that the peptides **3** containing the ΔAbu at the position 2 formed highly stable type I β-turns reinforced by H-bonds, normal or inverse depending on the stereochemistry of the Oxd^3. This result is particularly gratifying, since the formation of the ΔAbu-Oxd motif was somewhat unpredictable.

On the other hand, the data of the peptides **2** collected by ROESY/MD analyses confirmed a certain flexibility of the Phe^4 residue. This observation could explain the discrepancies in the determination of H-bonds by IR, VT-NMR, and NMR titration experiments. Nevertheless, the results highlighted the rigidity of the Ala-Oxd-Oxd portions; the rotation of the Ala and the two Oxd rings with respect to each other was not observed during the unrestrained MD simulations. The conformational homogeneity was correlated to the strong preference for all-*trans* conformations stabilized by the weak interactions between the Oxd carbonyls and the Hα protons of the preceding residues.

In particular, the heterochiral Oxd^2-D-Oxd^3 scaffold induced a stable and well defined type II β-turn, albeit the H-bond between the residues $i - i + 3$ was relatively loose. This ability to promote folded structures makes sense, and is consistent with the observations on previously described Oxd-peptides.[11,12] The oxazolidin-2-one ring can be regarded as a pseudo-Pro; it is well known that heterochiral linear oligopeptides including a Pro show higher propensity to fold compared to the homochiral sequences.[23,26]

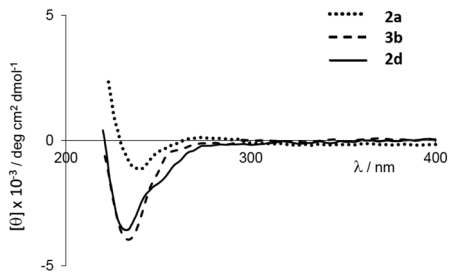


Fig. 5 ECD spectra recorded in dichloromethane at room temperature (**2a** dotted line, **3b** dashed line, **2d** full line).

Conclusions

In this paper we reported a straightforward, one-step procedure for constraining a peptide backbone without the need for a pre-formed scaffold. The cyclization of two consecutive β-hydroxy amino acid residues (L/D-Thr or *threo*-L/D-PhSer) embedded in *N*-arylsulfonyl tetrapeptide sequences afforded in excellent yields the peptides containing Oxd^2-L/D-Oxd^3 (**2**) or ΔAbu^2-L/D-Oxd^3 (**3**), according to the stereochemistry array of the precursors.

Conformational analyses of the model peptides revealed that the homochiral Oxd^2-Oxd^3 dipeptidomimetic induced an extended disposition of the backbone. The heterochiral sequences exerted a strong conformational control, giving rise to type II β-turns. The unrestrained MD simulations confirmed the rigidity of the conformations, since the rotation of the two Oxd rings with respect to each other was not observed during the simulations. On the other hand, the sequence ΔAbu^2-Oxd^3 induced highly stable β-turns of type I, stabilized by clear H-bonds.

For their ability to control the ϕ and ψ dihedral angles of the backbone as well as the χ dihedral angles of side chains, peptide mimetics based on the structures of the model peptides **2** and **3** might find applications in medicinal chemistry as 3D rigid scaffolds, in the field of foldamers, or as self-assembling scaffolds to form nanostructures.

Experimental

General methods

Unless stated otherwise, standard chemicals were obtained from commercial sources and used without further purification. Flash chromatography was performed on silica gel (230–400 mesh), using mixtures of distilled solvents. Analytical RP-HPLC was performed on an ODS column (4.6 μm particle size, 100 Å pore diameter, 250 μm, DAD 210 nm, from a 9 : 1 H_2O–CH_3CN to a 2 : 8 H_2O–CH_3CN in 20 min) at a flow rate of 1.0 mL min^{-1}, followed by 10 min at the same composition. Semi-preparative RP-HPLC was performed on a C18 column (7 μm particle size, 21.2 mm × 150 mm, from 8 : 2 H_2O–CH_3CN to 100% CH_3CN in 10 min) at a flow rate of 12 mL min^{-1}. Purities were assessed by analytical RP-HPLC under the above

reported conditions and elemental analysis. Chiral HPLC analysis was performed on a CHIRALPAK IC column (0.46 cm × 25 cm), 1 : 1 *n*-hexane–2-propanol, at 0.8 mL min^{-1}. Semi-preparative and analytical RP-HPLC of the peptide 14 was performed as reported above, with the addition of 0.1% TFA in the mobile phase. Elemental analyses were performed using a Thermo Flash 2000 CHNS/O analyzer. High-quality IR were obtained at 2 cm^{-1} resolution using a FT-IR spectrometer and 1 mm NaCl solution cell. ^{1}H-NMR spectra were recorded on a Varian instrument at 400 MHz, ^{13}C-NMR spectra at 100 MHz. Circular Dichroism (CD) spectra were recorded on a Jasco J-710 spectropolarimeter. The synthetic procedure by MW irradiation was performed using a microwave oven (Micro-SYNTH Microwave Labstation for Synthesis).

Synthesis of the peptides 1a–g

A stirred solution of the *N*-protected amino acid in 4 : 1 DCM–DMF (5 mL) was treated with HOBt (1.2 equiv.) and HBTU (1.2 equiv.), at r.t. and under inert atmosphere. After 5 min, the *C*-protected amino acid (1.1 equiv.) and DIPEA (2.4 equiv.) were added, and the mixture was stirred under inert atmosphere and under MW irradiation (150 W). After 10 min, the mixture was concentrated at reduced pressure, and the residue was diluted with EtOAc (25 mL). The solution was washed with 0.1 M HCl (5 mL), and a saturated solution of $NaHCO_3$ (5 mL). The organic layer was dried over Na_2SO_4 and the solvent was evaporated at reduced pressure. The crude peptides were analyzed by HPLC-MS analysis, and were used without further purification.

The intermediate *N*-Boc peptides were deprotected by treatment with 1 : 2 TFA–DCM (5 mL), while stirring at r.t. After 15 min, the solution was evaporated under reduced pressure, and the treatment was repeated. The residue was suspended in Et_2O (20 mL). The peptide–TFA salts were collected by centrifuge, and used for the next couplings without further purification (80–85% pure, Table 3).

Synthesis of Ts-Ala-Oxd-(5′-R-Oxd)-PheOMe (2a, c, d, f, g), Ts-Ala-ΔAbu-(5′-Me-Oxd)-PheOMe (3b, c).

DSC (0.73 mmol) was added to a stirred solution of **1** (0.33 mmol) in the solvent mixture of Table 1 (4 mL) followed by DIPEA (0.07 mmol) at r.t. and under inert atmosphere. After 3 h, the solvent was removed under reduced pressure, the residue was diluted with 0.1 M HCl (5 mL), and the mixture was extracted three times with DCM (5 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated at reduced pressure. The residue was purified by semi-preparative RP-HPLC (see General methods), giving **2** (85–93%, see Table 1, 94–97% pure by analytical RP-HPLC) or **3** (78–90%, see Table 1, 95–98% pure by analytical RP-HPLC), as waxy solids.

Ts-Ala-(5′-Me-Oxd^2)-(5′-Me-Oxd^3)-PheOMe (**2a**). IR (CH_2Cl_2) ν: 3406, 3335, 1789, 1736, 1703, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.43 (d, J = 6.8 Hz, 3H, AlaMe), 1.52 (d, J = 6.3 Hz, 6H, Oxd^{2}Me + Oxd^{3}Me), 2.43 (s, 3H, TsMe), 3.11 (dd, J = 5.5, 14.1 Hz, 1H, PheHβ), 3.15 (dd, J = 5.4, 14.1 Hz, 1H, PheHβ), 3.77 (s, 3H, OMe), 4.26 (d, J = 3.7 Hz, 1H, Oxd^{3}H$_4$), 4.58 (dq, J = 2.1, 6.3 Hz, 1H, Oxd^{2}H$_5$), 4.75 (dq, J = 3.7, 6.3 Hz, 1H, Oxd^{3}H$_5$), 4.85 (q, J = 7.6 Hz, 1H, PheHα), 5.22 (dq, J = 6.8, 10.4 Hz, 1H, AlaHα), 5.26 (d, J = 2.1 Hz, 1H, Oxd^{2}H$_4$), 5.39 (d, J = 10.4 Hz, 1H, AlaNH), 6.28 (d, J = 7.6 Hz, 1H, PheNH), 7.04–7.11 (m, 2H, PheArH), 7.21–7.26 (m, 3H, PheArH), 7.31 (d, J = 8.0 Hz, 2H, TsArH), 7.75 (d, J = 8.0 Hz, 2H, TsArH); ^{13}C-NMR (DMSO-d$_6$) δ: 18.5, 20.5, 21.3, 21.4, 36.9, 50.1, 52.5, 54.3, 61.0, 61.6, 74.7, 76.6, 126.0, 127.2, 128.6, 128.8, 129.6, 130.1, 137.0, 138.2, 143.5, 152.0, 153.2, 167.4, 167.7, 171.7, 172.4; ES-MS (m/z) 659.3 [M + 1], calcd 659.2; Elem. Anal. for $C_{30}H_{34}N_4O_{11}S$, calcd: C 54.70, H 5.20, N 8.51, S 4.87; found: C 54.67, H 5.17, N 8.45, S 4.81%.

Ts-Ala-ΔAbu-(5′-Me-Oxd)-PheOMe (**3b**). IR (CH_2Cl_2) ν: 3407, 3318, 1789, 1748, 1708 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.23 (d, J = 6.8 Hz, 3H, AlaMe), 1.43 (d, J = 6.0 Hz, 3H, OxdMe), 1.79 (d, J = 7.2 Hz, 3H, ΔAbuMe), 2.43 (s, 3H, TsMe), 3.03 (dd, J = 8.2, 13.8 Hz, 1H, PheHβ), 3.23 (dd, J = 5.4, 13.8 Hz, 1H, PheHβ), 3.71 (s, 3H, OMe), 3.85 (quint, J = 7.2 Hz, 1H, AlaHα), 4.36–4.41 (m, 2H, OxdH$_{4,5}$), 4.84 (q, J = 8.2 Hz, 1H, PheHα), 5.92 (d, J = 6.8 Hz, 1H, AlaNH), 6.04 (q, J = 7.2 Hz, 1H, ΔAbuHβ), 7.14–7.22 (m, 2H, PheArH), 7.24–7.34 (m, 5H, PheArH + TsArH), 7.64 (d, J = 8.0 Hz, 1H, PheNH), 7.77 (d, J = 7.6 Hz, 2H, TsArH), 8.66 (s, 1H, ΔAbuNH); ^{13}C-NMR (DMSO-d$_6$) δ: 12.8, 19.4, 20.3, 37.0, 51.9, 52.4, 54.1, 62.1, 75.0, 120.5, 126.9, 127.2, 128.8, 129.6, 130.1, 130.5, 134.1, 137.1, 138.7, 143.2, 151.8, 168.6, 170.8, 170.9, 171.9; ES-MS (m/z) 615.4 [M + 1], calcd 615.2; Elem. Anal. for $C_{29}H_{34}N_4O_9S$, calcd: C 56.67, H 5.58, N 9.11, S 5.22; found: C 56.78, H 5.56, N 9.08, S 5.18%.

Ts-Ala-(5′-Me-Oxd^2)-D-(5′-Me-Oxd^3)-PheOMe (**2c**) IR (CH_2Cl_2) ν 3406, 3347, 1793, 1736, 1707 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.33 (d, J = 6.6 Hz, 3H, AlaMe), 1.47 (d, J = 6.2 Hz, 3H, Oxd^{2}Me), 1.53 (d, J = 6.4 Hz, 3H, Oxd^{3}Me), 2.42 (s, 3H, TsMe), 3.01 (dd, J = 6.4, 14.1 Hz, 1H, PheHβ), 3.20 (dd, J = 6.4, 14.1 Hz, 1H, PheHβ), 3.77 (s, 3H, OMe), 4.29 (d, J = 4.8 Hz, 1H, Oxd^{3}H$_4$), 4.53 (dq, J = 2.4, 6.4 Hz, 1H, Oxd^{2}H$_5$), 4.64 (quint, 1H, J = 6.2 Hz, Oxd^{3}H$_5$), 4.82 (q, J = 6.4 Hz, 1H, PheHα), 5.11 (dq, J = 6.8, 13.6 Hz, 1H, AlaHα), 5.24 (d, J = 2.4 Hz, 1H, Oxd^{2}H$_4$), 5.44 (d, J = 10.0 Hz, 1H, AlaNH), 6.55 (d, J = 7.6 Hz, 1H, PheNH), 7.09 (d, J = 7.2 Hz, 2H, PheArH), 7.24–7.31 (m, 5H, PheArH + TsArH), 7.73 (d, J = 7.6 Hz, 2H, TsArH); ^{13}C-NMR (DMSO-d$_6$) δ: 18.5, 20.4, 20.5, 21.4, 37.4, 50.3, 52.6, 53.8, 61.3, 62.0, 69.4, 70.1, 127.2, 128.4, 128.7, 129.8, 130.1, 137.4, 138.3, 143.5, 152.3, 153.3, 166.5, 167.1, 171.7, 172.0; ES-MS (m/z) 659.4 [M + 1],

Table 3 RP-HPLC and ES-MS analyses of the linear peptides **1a–f**

1	ES-MS m/z [M + 1] *vs.* calcd	Purity[a] (%)	1	ES-MS m/z [M + 1] *vs.* calcd	Purity[a] (%)
a	607.3/607.2	85	**e**	607.2/607.2	84
b	607.2/607.2	83	**f**	669.3/669.3	84
c	607.3/607.2	81	**g**	669.4/669.3	83
d	607.1/607.2	80			

[a] Determined by analytical RP-HPLC, see General methods.

calcd 659.2; Elem. Anal. for $C_{30}H_{34}N_4O_{11}S$, calcd: C 54.70, H 5.20, N 8.51, S 4.87; found: C 54.60, H 5.14, N 8.44, S 4.81%.

Ts-Ala-ΔAbu-D-(5′-Me-Oxd)-PheOMe (**3c**). IR (CH_2Cl_2) ν: 3400, 3334, 1781, 1744, 1707 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.28 (d, J = 7.2 Hz, 3H, AlaMe), 1.54 (d, J = 6.0 Hz, 3H, OxdMe), 1.89 (d, J = 7.2 Hz, 3H, ΔAbuMe), 2.46 (s, 3H, TsMe), 3.09 (dd, J = 9.0, 13.8 Hz, 1H, PheHβ), 3.18 (dd, J = 6.2, 13.8 Hz, 1H, PheHβ), 3.72 (s, 3H, OMe), 3.87 (m, 1H, AlaHα), 4.45 (d, J = 7.2 Hz, 1H, $OxdH_4$), 4.59 (quint, J = 6.5 Hz, 1H, $OxdH_5$), 4.79 (q, J = 8.4 Hz, 1H, PheHα), 5.66 (br.d, 1H, AlaNH), 6.27 (q, J = 6.6 Hz, 1H, ΔAbuHβ), 7.21 (d, J = 8.4, 2H, PheArH), 7.23–7.33 (m, 3H, PheArH), 7.35 (d, J = 7.8 Hz, 2H, TsArH), 7.80 (d, J = 7.8 Hz, 2H, TsArH), 7.98 (d, J = 8.0 Hz, 1H, PheNH), 8.28 (s, 1H, ΔAbuNH); ^{13}C-NMR (DMSO-d_6) δ: 12.8, 16.9, 21.5, 25.7, 37.8, 52.8, 54.7 56.9, 62.0, 75.2, 121.9, 126.0, 127.0, 128.7, 129.9, 132.0, 134.4, 136.3, 143.0, 154.8, 168.4, 170.8, 171.6; ES-MS (m/z) 615.4 [M + 1], calcd 615.2; Elem. Anal. for $C_{29}H_{34}N_4O_9S$, calcd: C 56.67, H 5.58, N 9.11, S 5.22; found: C 56.60, H 5.52, N 9.02, S 5.15%.

Ts-Ala-(5′-Me-Oxd^2)-D-(5′-Me-Oxd^3)-D-PheOMe (**2d**). IR (CH_2Cl_2) ν: 3409, 1776, 1747, 1707, 1608, 1420 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.40 (d, J = 7.0 Hz, 3H, AlaMe), 1.46 (d, J = 6.0 Hz, 3H, Oxd^3Me), 1.58 (d, J = 6.3 Hz, 3H, Oxd^2Me), 2.44 (s, 3H, TsMe), 3.05 (dd, J = 8.3, 13.9 Hz, 1H, PheHβ), 3.17 (dd, J = 5.3, 13.9 Hz, 1H, PheHβ), 3.74 (s, 3H, OMe), 4.29–4.37 (m, 2H, $Oxd^3H_{4,5}$), 4.58 (dq, J = 3.5, 6.3 Hz, 1H, Oxd^2H_5), 4.88 (ddd, J = 5.3, 8.3, 8.5 Hz, 1H, PheHα), 5.16 (dq, J = 7.0, 10.8 Hz, 1H, AlaHα), 5.42 (d, J = 3.5 Hz, 1H, Oxd^2H_4), 5.61 (d, J = 10.8 Hz, 1H, AlaNH), 6.63 (d, J = 8.5 Hz, 1H, PheNH), 7.14 (d, J = 6.6 Hz, 2H, PheArH), 7.22–7.37 (m, 5H, PheArH + TsArH), 7.76 (d, J = 8.0 Hz, 2H, TsArH); ^{13}C-NMR ($CDCl_3$) δ: 18.9, 20.6, 20.9, 21.5, 37.9, 51.1, 52.6, 53.4, 61.1, 62.4, 74.2, 76.0, 127.2, 127.4, 128.6, 129.3, 129.7, 135.6, 136.9, 143.8, 151.1, 152.0, 165.7, 168.1, 171.4, 173.8; ES-MS (m/z) 659.3 [M + 1], calcd 659.2; Elem. Anal. for $C_{30}H_{34}N_4O_{11}S$, calcd: C 54.70, H 5.20, N 8.51, S 4.87; found: C 54.65, H 5.26, N 8.50, S 4.82%.

Ts-Ala-(5′-Me-Oxd^2)-(5′-Ph-Oxd^3)-PheOMe (**2f**). IR (CH_2Cl_2) ν: 3407, 3350, 1790, 1740, 1699, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.41 (d, J = 7.2 Hz, 3H, AlaMe), 1.55 (d, J = 6.2 Hz, 6H, Oxd^2Me), 2.43 (s, 3H, TsMe), 3.12 (d, J = 5.6 Hz, 2H, PheHβ), 3.78 (s, 3H, OMe), 4.53 (d, J = 4.4 Hz, 1H, Oxd^3H_4), 4.66 (dq, J = 2.0, 6.2 Hz, 1H, Oxd^2H_5), 4.90 (q, J = 7.6 Hz, 1H, PheHα), 5.24 (dq, J = 7.6, 10.8 Hz, 1H, AlaHα), 5.35 (d, J = 2.0 Hz, 1H, Oxd^2H_4), 5.41 (d, J = 10.8 Hz, 1H, AlaNH), 5.57 (d, J = 4.4 Hz, 1H, Oxd^3H_5), 6.31 (d, J = 7.7 Hz, 1H, PheNH), 7.04–7.12 (m, 2H, PheArH), 7.20–7.30 (m, 5H, PheArH + 5′-PhArH), 7.31 (d, J = 8.0 Hz, 2H, TsArH), 7.39–7.44 (m, 3H, 5′-PhArH), 7.77 (d, J = 8.0 Hz, 2H, TsArH); ^{13}C-NMR ($CDCl_3$) δ: 19.5, 21.1, 21.5, 37.2, 50.6, 52.7, 53.7, 61.2, 65.8, 73.8, 79.0, 125.2, 127.3, 127.6, 129.1, 129.4, 129.5, 129.9, 130.1, 135.9, 136.6, 143.8, 151.1, 152.3, 166.1, 167.4, 173.1, 173.4; ES-MS (m/z) 721.2 [M + 1], calcd 721.2; Elem. Anal. for $C_{35}H_{36}N_4O_{11}S$, calcd: C 58.32, H 5.03, N 7.77, S, 4.45; found: C 58.29, H 5.09, N 7.80, S, 4.40%.

Ts-Ala-(5′-Me-Oxd^2)-D-(5′-Ph-Oxd^3)-PheOMe (**2g**). IR (CH_2Cl_2) ν: 3409, 3345, 1779, 1730, 1700, 1656 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.30 (d, J = 7.3 Hz, 3H, AlaMe), 1.52 (d, J = 6.4 Hz, 3H, Oxd^2Me), 2.43 (s, 3H, TsMe), 3.03 (dd, J = 7.2, 14.4 Hz, 2H, PheHβ), 3.23 (dd, J = 6.0, 14.4 Hz, 2H, PheHβ), 3.76 (s, 3H, OMe), 4.53–4.61 (m, 2H, Oxd^2H_5 + Oxd^3H_4), 4.88 (q, J = 7.6 Hz, 1H, PheHα), 5.13 (dq, J = 7.6, 10.8 Hz, 1H, AlaHα), 5.29 (d, J = 2.4 Hz, 1H, Oxd^2H_4), 5.50–5.58 (m, 2H, AlaNH + Oxd^3H_5), 6.63 (d, J = 7.7 Hz, 1H, PheNH), 7.04–7.11 (m, 2H, PheArH), 7.15–7.20 (m, 2H, 5′-PhArH), 7.20–7.25 (m, 3H, PheArH), 7.31 (d, J = 8.0 Hz, 2H, TsArH), 7.39–7.43 (m, 3H, 5′-PhArH), 7.76 (d, J = 8.0 Hz, 2H, TsArH); ^{13}C-NMR ($CDCl_3$) δ: 19.3, 20.6, 21.5, 37.6, 50.7, 53.3, 53.5, 60.8, 63.4, 71.8, 79.0, 125.3, 127.4, 128.7, 129.2, 129.5, 129.7, 130.1, 134.7, 135.7, 136.7, 143.6, 151.3, 152.3, 165.4, 167.0, 171.2, 173.1; ES-MS (m/z) 721.3 [M + 1], calcd 721.2; Elem. Anal. for $C_{35}H_{36}N_4O_{11}S$, calcd: C 58.32, H 5.03, N 7.77, S, 4.45; found: C 58.22, H 5.07, N 7.71, S, 4.49%.

Conformational analysis

IR analyses. Infrared spectra were obtained at 2 cm^{-1} resolution using a 1 mm NaCl solution cell and a FT-IR spectrometer (64 scans). All spectra were obtained in 3 mM solutions in dry CH_2Cl_2 at 297 K. The compounds were dried *in vacuo*, and all the sample preparations were performed in a inert atmosphere.

Circular dichroism. ECD spectra were recorded from 200 to 400 nm at 25 °C. 1 mM solutions were made up in spectral grade solvents and run in a 0.1 cm quartz cell. Data are reported in molar ellipticity [θ] (deg cm^2 $dmol^{-1}$).

NMR analyses. ^{1}H-NMR spectra were recorded at 400 MHz in 5 mm tubes, using 0.01 M peptide at room temperature. Solvent suppression was performed by the solvent presaturation procedure implemented in Varian (PRESAT). ^{13}C-NMR spectra were recorded at 100 MHz. Chemical shifts are reported as δ values. The unambiguous assignment of ^{1}H-NMR resonances was performed by 2D gCOSY, HMBC, and HSQC. gCOSY experiments were conducted with a proton spectral width of 3103 Hz. VT-^{1}H-NMR experiments were performed over the range of 298–348 °K. 2D spectra were recorded in the phase sensitive mode and processed using a 90°-shifted, squared sine-bell apodization. 2D ROESY experiments were recorded in 8 : 2 DMSO-d_6–H_2O, with a 250 ms mixing time with a proton spectral width of 3088 Hz. Peaks were calibrated on DMSO.

ROESY and molecular dynamics. Only ROESY-derived constraints were included in the restrained molecular dynamics. Cross-peak intensities were classified very strong, strong, medium, and weak, and were associated with distances of 2.2, 2.6, 3.0, and 4.5 Å, respectively. Geminal couplings and other obvious correlations were discarded. For the absence of Hα(i, i + 1) ROESY cross peaks, all of the ω bonds were set at 180° (force constant: 16 kcal mol^{-1} $Å^{-2}$). The restrained MD simulations were conducted using the AMBER force field in a 30 × 30 × 30 Å box of standard TIP3P models of equilibrated water.[31] All water molecules with atoms that come closer than 2.3 Å to a solute atom were eliminated. A 100 ps simulation at 1200 °K was used for generating 50 random structures that were subsequently subjected to a 50 ps restrained MD with a 50% scaled force field at the same temperature, followed by

50 ps with full restraints (distance force constant of 7 kcal mol^{-1} $Å^{-2}$), after which the system was cooled in 20 ps to 50 ° K. H-bond interactions were not included, nor were torsion angle restraints. The resulting structures were minimized with 3000 cycles of steepest descent and 3000 cycles of conjugated gradient (convergence of 0.01 kcal $Å^{-1}$ mol^{-1}). The backbones of the structures were clustered by the RMSD analysis module of HyperChem.[32] Unrestrained MD simulation was performed in a 30 × 30 × 30 Å box of standard TIP3P water for 10 ns at 298 °K, at constant temperature and pressure (Berendsen scheme,[33] bath relaxation constant of 0.2). For 1–4 scale factors, van der Waals and electrostatic interactions are scaled in AMBER to half their nominal value. The integration time step was set to 0.1 fs.

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