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Strained-Cyclophane-Induced β-Turn Template: Design, Synthesis, and Spectroscopic Characterization

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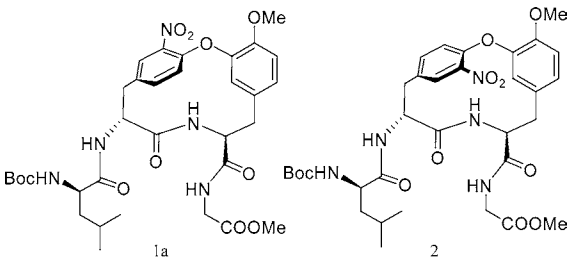
ABSTRACT



Three tetrapeptides incorporating a 14-membered (R_{i+1}, S_{i+2}) cycloisodityrosine at the $i + 1$ and $i + 2$ positions were designed and synthesized. Conformational analysis by ^{1}H NMR and CD spectra as well as molecular modeling indicated that they all adopt a β-turn conformation. While the CD spectrum of compound 2 is characteristic of the typical type-II β-turn (maximum at ∼200 nm and a minimum at ∼220 nm), that of 1a (atropisomer of 2) is opposite in sign to the expected spectrum of the type-II β-turn.

β-Turns are a subset of reverse turns and consist of a tetrapeptide sequence in which the $\alpha_{Ci}-\alpha_{Ci+3}$ distance is shorter than 7 Å.[1] Such turns are often stabilized by an intramolecular hydrogen bond between the carboxyl oxygen of the *i* residue and the amide proton of the $i + 3$ residue, which leads to the formation of a 10-membered ring-type structure. β-Turns are often located on the protein surface and hence play important roles in the molecular recognition events of biological systems.[2] A great deal of effort has therefore been focused on the design and synthesis of small constrained mimetics of this turn pattern.[3] Two types of β-turn have been devised and synthesized; they are commonly referred to as internal and external mimetics, respectively.[3d] While macrocycles have been used for the construction of internal β-turn mimetics,[4] they have only been rarely used for inducing an external β-turn. In this regard, Katzenellenbogen has demonstrated that a 10-membered lactam is capable of restricting the φ and ψ torsion angles of a tetrapeptide to those found in a type-I β-turn.[5] Herein, we report the design, synthesis, and spectroscopic charac-

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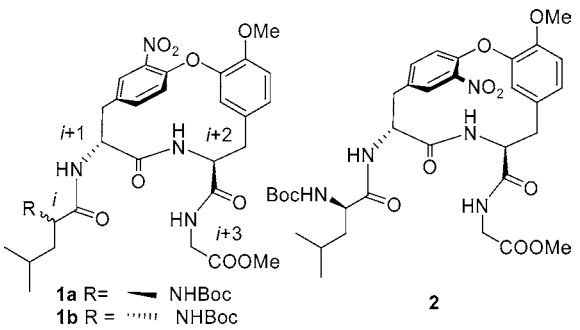

Figure 1. Structure of tetrapeptides incorporating cycloisodityrosine unit at $i + 1$ and $i + 2$ positions.

terization of a cyclophane-induced external type-II β-turn structure (**1** and **2**) and document the effect of planar chirality on the circular dichroism (CD) spectra of these turns (Figure 1).

RA-VII (**3**, Figure 2), a natural product with potent antitumor activity, was the departure point for the present research program.[6] Both X-ray and solution NMR spectroscopic analysis of RA-VII indicate the presence of a typical type-II β-turn structure for the major conformer. Since it is well-known that 18-membered cyclic hexapeptides are prone to adopting a turn-extended-turn conformation,[7] it is thus not unexpected that RA-VII contains a β-turn motif within the 18-membered ring. On the other hand, it was unknown whether the 14-membered *m*,*p*-cyclophane alone (**4** or its diastereomer **5**) was able to induce an external β-turn at the outset of this work. Inspection of the X-ray structure of (S_{i+1},S_{i+2})-cyclophane (**4**) and (R_{i+1},S_{i+2})-cyclophane (**5**, Figure 2)[8] indicated that only the nonnatural (R_{i+1},S_{i+2})-stereomer **5** was capable of acting as a β-turn inducer.[9] To gain further insight into the conformational properties of these two cyclophanes, molecular modeling of compounds **6** and **7** was carried out. In accord with our speculation, only the (R_{i+1},S_{i+2})-**7**, not the (S_{i+1},S_{i+2})-**6**, adopted a β-turn conformation. Indeed, the values of the torsion angles of the lower energy conformer of **7**, ϕ (i+1) 70.8, ψ (i+1) −117.1, ϕ (i+2) −79.1, ψ (i+2) −7.6, corresponded nicely to that of an ideal type-II′ β-turn.

To verify these computational results and to probe the influence of the planar chirality on the conformation of the tetrapeptide, compounds **1a**,**b** and **2** were synthesized via a

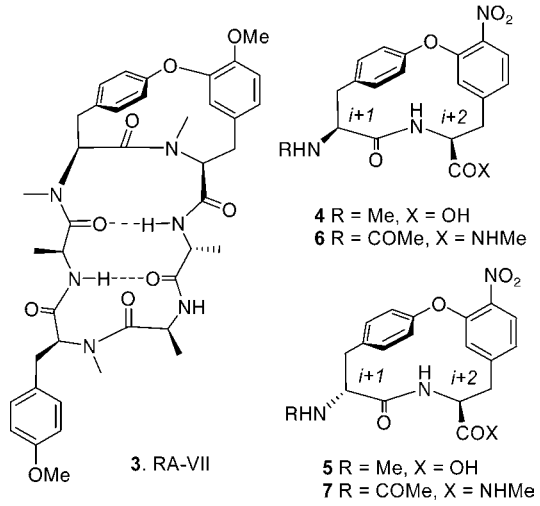

Figure 2. Structure of RA-VII and isodityrosine.

size-selective ring forming process based on the intramolecular S_NAr reaction (Scheme 1). Thus, cycloetherification of dipeptide **8** (DMSO K_2CO_3, molecular sieve 3 Å, room temperature) gave, after methylation of the remaining phenol function, the corresponding 14-membered *m*,*p*-cyclophane as two separable atropisomers **9** and **10** in 45% yield.[10] The observed NOE correlation between protons H_a-H_b for **9** and H_a-H_c for **10**, respectively, is indicative of their respective planar chirality.[11] The diastereomerically pure atropisomers **9** and **10** were converted to the corresponding tetrapeptides **1a** and **2**, respectively, following the standard deprotection-coupling protocol. Tetrapeptides **1b** incorporating a (*S*)-NHBoc leu at the *N*-terminal were synthesized following the same synthetic sequence from cyclophane **9**.

Conformational analyses of **1a**,**b** and **2** were undertaken to probe if they could indeed access β-turn conformations. NMR studies of these compounds in DMSO-d_6 indicated the presence of a sole conformer at room temperature. Spectroscopic assignments of all protons were made on the basis of COSY and ROESY spectra. Temperature coefficients ($\Delta\delta/\Delta T$) were measured by recording 10 ^{1}H NMR spectra between 298 and 343 K with an increment of 5 °C in DMSO-d_6 and are summarized in Table 1. The relatively low-temperature coefficient values for the NH_{i+3} of compounds **1a**, **2**, and **1b** ($\Delta\delta/\Delta T$ = −2.77, −2.95, and −3.68 ppb/K, respectively) indicated that these protons were engaged in intramolecular hydrogen bonding. Significant NOE connectivities between the NH_{i+2} and NH_{i+3}; NH_{i+2} and αCH_{i+1} and the lack of correlation between protons NH_{i+1} and NH_{i+2} were indicative of type II-like β-turn conformations. The observation of an NOE cross-peak between protons NH_{i+2}

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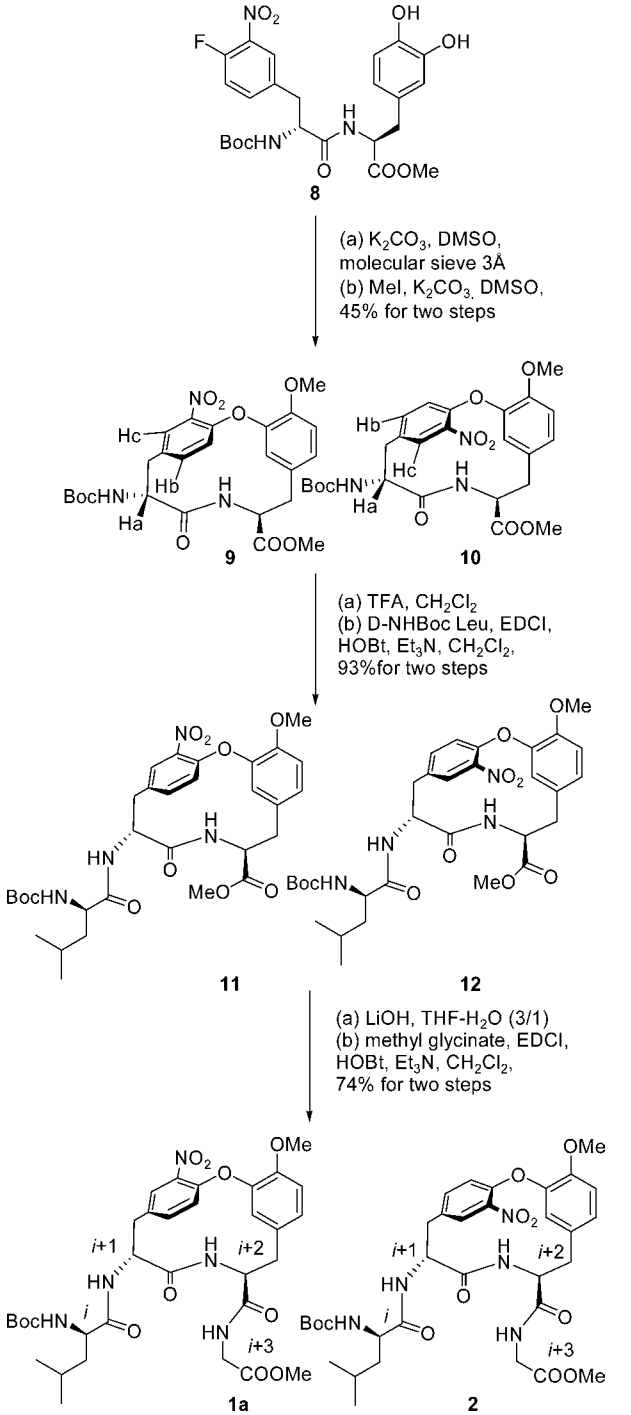
(11) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons Inc.: New York, 1994; Chapter 14.

Scheme 1. Synthesis of Tetrapeptides **1a** and **2**



and αCH_{i+1} also indicated the trans configuration of the central amide bond in contrast to the *cis*-configuration for RAs.

Circular dichroism CD spectra have been used extensively for the characterization of peptide conformations. The presence of two aromatic rings in the cyclophanes **1** and **2**[12] may significantly influence or dominate the expected CD spectrum of a given peptide and thus provide inconclusive information. Furthermore, these compounds have a plane of chirality that could modify the CD spectrum. Keeping this in mind, different solvents (DMSO, DMSO−H_2O, MeOH−H_2O, and MeCN) were used for recording the CD spectra of **1a**,**b** and **2** and those recorded in acetonitrile are shown in Figure 3.[13]

Table 1. Temperature Coefficients ($\Delta\delta/\Delta T$, ppb/K) for the NH Protons of Compounds **1a**,**b** and **2**[a]

	NH_{i+3}	NH_{i+2}	NH_{i+1}	NH_i
1a	−2.77	−4.65	−8.36	−6.26
1b	−3.68	−4.54	−9.81	−11.63
2	−2.95	−4.07	−8.42	−6.21

[a] ^{1}H NMR spectra were recorded on a Bruker Avance-600 (600 MHz).

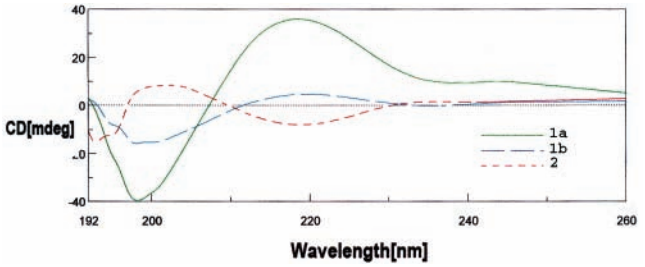


Figure 3. CD Spectra of compounds **1a**,**b** and **2**.

In this solvent, the CD spectrum for compound **2** is characteristic of a typical type II β-turn, it exhibits a maximum at ∼200 nm and a minimum at ∼220 nm. On the other hand, the CD spectra for compound **1a**, the atropisomer of **2**, contained a minimum at ∼200 nm and a maximum at ∼220 nm, exactly the opposite of the expected spectrum of a type-II β-turn. Since it is known that atropisomers can display CD spectra opposite in sign,[14] we hypothesized that this might be the case for the β-turn motifs. CD spectra similar to **1a** were observed for compound **1b**, indicating that this trend may be a general phenomenon. The maximum absolute value of the molar ellipticity for **1a** was higher than for **2** and **1b**, indicating that both the planar chirality and the absolute configuration of amino acid *i* might influence the conformational flexibility of the tetrapeptide, with **1a** being less flexible. This conclusion is in accord with the lower $\Delta\delta/\Delta T$ value of NH_{i+3} for compound **1a**. Computational studies of compounds **1a** and **2** indicate that the low-energy conformations adopted by these compounds are indeed β-turns (Monte Carlo random search, optimized by Macromodel program, version 5.5, AMBER

(12) It is worth noting that the 1,4-disubstituted benzene ring in compounds **1a**,**b** and **2** was deformed due to the high ring strain associated with this *m*,*p*-cyclophane.

(13) Solvent effect on CD spectra: Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

(14) For example, see: Jeffs, P. W.; Chan, G.; Mueller, L.; Debrosse, C.; Webb, L.; Sitrin, R. *J. Org. Chem.* **1986**, *57*, 4272−4278.

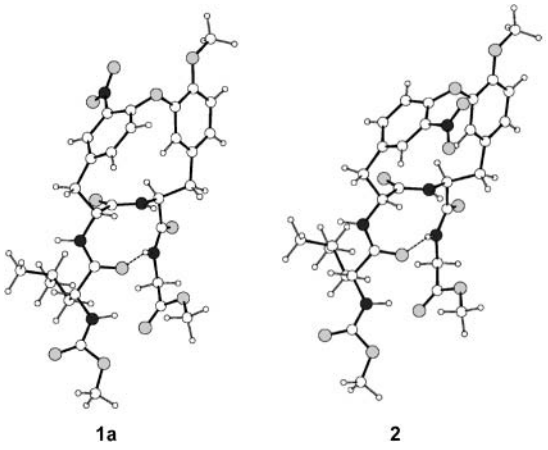


Figure 4. Low energy conformers of compounds **1a** and **2**.

force field and GB/SA water solvation, Figure 4, cf. Supporting Information).

In summary, we have demonstrated that (R_{i+1},S_{i+2})-cycloisodityrosine can act effectively as an external β-turn inducer and we have documented for the first time, a situation in which two β-turns, with identical amino acid residues and central carbon chiralities, gave CD spectra opposite in sign due to the presence of a remote planar chirality. The cycloisodityrosine **9** or **10** can be considered as a promising scaffold for the design and synthesis of libraries of short oligomers with a well-defined secondary structure.

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Supporting Information Available: Spectroscopic data for compounds **1a**,**b**, **2**, and **8**−**10** and conformational studies (**1a**,**b** and **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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