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Synthesis of [Tyr-5-Ψ(CH_2NMe)-Tyr-6]RA-VII, a reduced peptide bond analogue of RA-VII, an antitumor bicyclic hexapeptide

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ABSTRACT

A reduced peptide bond analogue of RA-VII, [Tyr-5-Ψ(CH_2NMe)-Tyr-6]RA-VII (**3**), was designed and synthesized. The key reduced cycloisodityrosine unit was prepared by reduction of the cycloisodityrosine derived from natural RA-VII, followed by connection with the tetrapeptide segment to afford a hexapeptide. Subsequent macrocyclization of the hexapeptide with FDPP under dilute conditions gave **3**. Analogue **3** showed cytotoxic activity against P-388 cells, but its activity was much weaker than that of parent peptide RA-VII.

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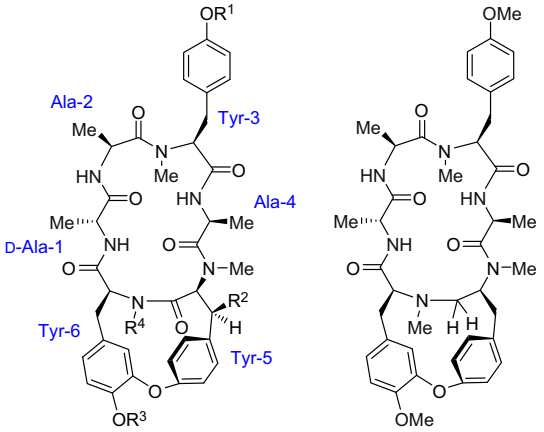
RA-VII (**1**), which was isolated from *Rubia cordifolia* L. and *R. akane* Nakai (Rubiaceae),[1,2] and bouvardin (NSC 259968, **2**), which was isolated from *Bouvardia ternifolia* (Cav.) Schltdl. (Rubiaceae),[3] are bicyclic hexapeptides that are structurally characterized by the presence of a unique and strained 14-membered cycloisodityrosine unit in the molecule (Fig. 1). These peptides have promising antitumor activity, and peptide **1** had undergone phase I clinical trials as an anticancer drug in Japan.[4] The antitumor activity of these peptides is believed to be due to the inhibition of protein synthesis through the interaction with eukaryotic ribosomes.[5,6] Peptide **1** has been shown also to induce conformational changes in F-actin, stabilizing actin filaments and inducing G2 arrest.[7] During the phase I clinical trials of **1**, we encountered formulation problems associated with its poor solubility in water. Therefore, the key consideration in our design of new peptide **1** analogues was to improve the solubility of **1**. In this Letter, we describe the synthesis of a novel analogue of RA-VII, [Tyr-5-Ψ(CH_2NMe)-Tyr-6]RA-VII (**3**), and the evaluation of its cytotoxicity.

Modifications of the peptide backbone are often conducted for the synthesis of bioactive peptide analogues.[8] One example is the replacement of the amide bond with a reduced peptide bond to introduce a basic nitrogen into the peptide backbone.[9] Compared with the original peptide, such a reduced peptide bond analogue is expected to have improved aqueous solubility through the formation of an acid salt, and thus may simplify formulation and in vivo use. We considered that the replacement of the peptide bond between the Tyr-5 and Tyr-6 residues of RA-VII (**1**) with a reduced peptide bond, –CH_2NMe–, as in analogue **3**, would little affect the gross conformation of the molecule because this peptide bond would be shared by both the 14-membered and 18-membered rings and the rotation about this bond would be



	R^1	R^2	R^3	R^4
RA-VII (**1**):	Me	H	Me	Me
bouvardin (**2**):	Me	OH	H	Me
4:	Me	H	Me	H
RA-II (**5**):	H	H	Me	Me

Figure 1. Structures of RA-series peptides and bouvardins.

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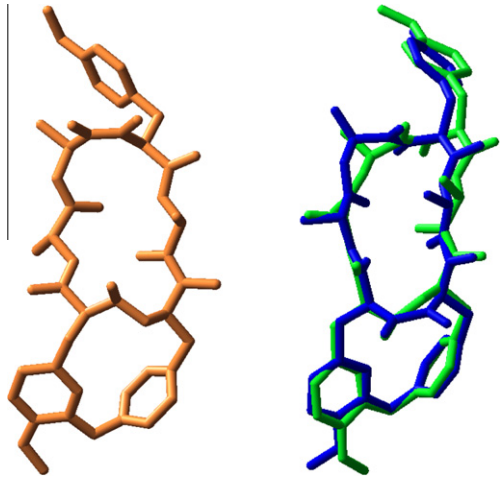

Figure 2. Energy-minimized structures of **3** with a *cis* (orange) or a *trans* (green) amide bond between residues 2 and 3. The latter is superimposed with the crystal structure of RA-II (**5**, blue).

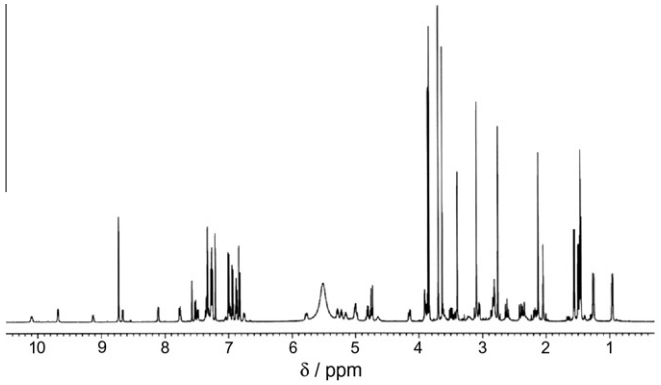

Figure 4. ^{1}H NMR (500 MHz) spectrum of analogue **3**.

restricted by the structural nature of the cycloisodityrosine unit that would incorporate a *meta*-substituted phenyl ring and a *para*-substituted phenyl ring. Indeed, N^{29}-desmethyl-RA-VII (**4**), which lacks an *N*-methyl group at the Tyr-6 residue, is known to still adopt an unusual *cis* configuration at this amide bond, thus retaining the conformational features of **1** and expressing potent cytotoxicity.[10] In analogue **3**, the Monte Carlo conformational search gave two distinctive conformers characterized by a *cis* or a *trans* amide bond between the Ala-2 and Tyr-3 residues, and the former was slightly more stable ($\Delta E = -1.1$ kcal/mol) than the latter (Fig. 2).[11] Comparison of the structure of the latter conformer with the X-ray crystal structure of RA-II (**5**) indicated that their 3D structures were similar (Fig. 2). Thus, it was thought that analogue **3** might retain potent antitumor activity.

Analogue **3** was synthesized by the following procedure (Fig. 3). Key reduced cycloisodityrosine unit **6**, corresponding to Tyr-5 and Tyr-6 residues, was prepared by selective reduction of the amide bond of cycloisodityrosine **7**, which was obtained by the degradation of natural RA-VII (**1**).[12] The Boc group of **7** was removed by treatment with trifluoroacetic acid (TFA) and the product was then treated with 1.2 molar equiv of borane–tetrahydrofuran complex in tetrahydrofuran (THF) at room temperature for 3 days. Aqueous workup and subsequent treatment of the reaction mixture with Boc_2O gave desired reduced cycloisodityrosine **6** in 49% yield. The tetrapeptide segment corresponding to residues 1–4 was prepared from previously synthesized tripeptide **8**.[12b] Extension at the N-terminus of tripeptide **8** by Boc-D-Ala-OH gave tetrapeptide **9**, and subsequent ester hydrolysis of it afforded acid **10**. After removal of the Boc group, compound **6** was coupled with tetrapeptide carboxylic acid **10** in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBt) to provide hexapeptide **11** in 68% yield. The benzyl-protecting group at the C-terminus and the Boc group at the N-terminus of peptide **11** were removed sequentially by catalytic hydrogenolysis, followed by treatment with HCl. The resulting deprotected hexapeptide was then subjected to macrocyclization under dilute conditions (0.0027 M) in *N,N*-dimethylformamide (DMF) at 0 °C for 4 days with pentafluorophenyl diphenylphosphinate (FDPP) and *N,N*-diisopropylethylamine (DIEA) to afford analogue **3** in 47% yield from **11**.

The solution structure of analogue **3** was analyzed by NMR spectroscopy.[13] The ^{1}H NMR spectrum of analogue **3** in pyridine-d_5 at 300 K showed the presence of three conformers in 54:44:2 ratio (Fig. 4). In the NOESY spectrum, the most populated conformer with population 54% (conformer-I, Fig. 5) showed NOE correlations between Ala-2 H_α/Tyr-3 NMe, Tyr-3 NMe/Tyr-3 H_α, Ala-4 H_α/Tyr-5

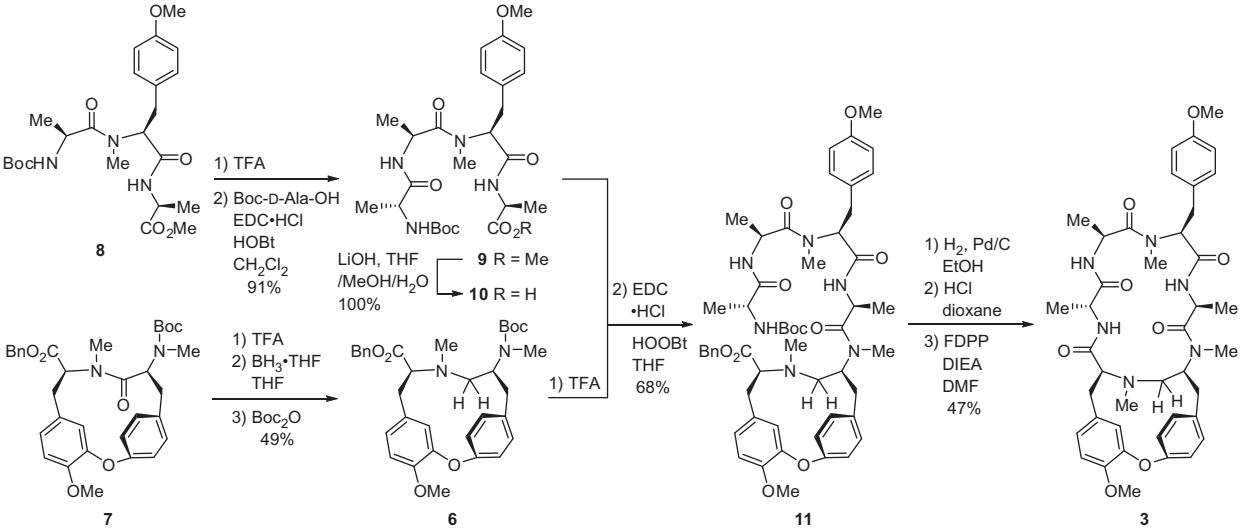

Figure 3. Synthesis of analogue **3**.

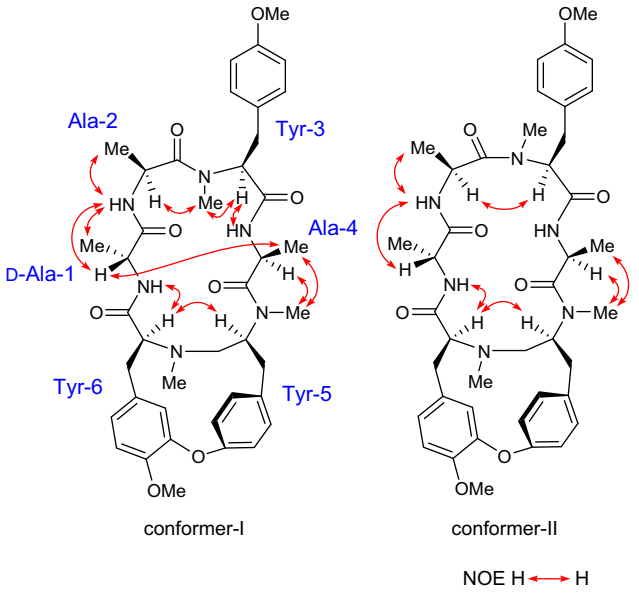

Figure 5. Key NOE correlations of analogue **3**.

NMe, and Ala-4 $H_{3\beta}$/Tyr-5 NMe, indicating that the amide configurations between Ala-2 and Tyr-3 and between Ala-4 and Tyr-5 are both *trans*. The correlation between Tyr-5 H_α/Tyr-6 H_α demonstrated the proximity of these protons, suggesting that the two carbon atoms, Tyr-5 C_α and Tyr-6 C_α, are in a *gauche* or nearly an *eclipse* arrangement as expected from the Monte Carlo conformational search of **3** (Fig. 2). Further, an NOE correlation was observed between D-Ala-1 H_α/Ala-4 $H_{3\beta}$. This correlation is usually observed when this series of natural peptides adopt the conformation as depicted in the crystal structure of RA-II (**5**) in Figure 2. Accordingly, this conformer seemed to have very similar structural features to the calculated structure of **3** shown in green in Figure 2.

The conformer with population 44% (conformer-II, Fig. 5) differed from conformer-I in that it adopted a *cis* amide configuration between Ala-2 and Tyr-3 (Fig. 5). The structure of the third conformer with population 2% could not be determined due to weak signal intensity.

Analogue **3** and, as reference, peptide **1**, were evaluated for their cytotoxicity to P-388 leukemia cells, and their IC_{50} values were 5.0 and 0.0043 μg/mL, respectively.[14] In spite of the resemblance of the 3D structure of the most populated conformer of analogue **3** in pyridine-d_5 solution to that of the active conformation of this series of peptides as represented by the crystal structure of RA-II (**5**),[15] analogue **3** showed only 1/1200 of the activity of peptide **1**. This unexpectedly weak activity of analogue **3** may be due to subtle differences in the peptide backbone conformation and/or the lack of amide carbonyl oxygen at Tyr-5 in analogue **3**.

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11. The software used for this calculation was MacroModel ver. 7.0 (Schrödinger Inc.). The Monte Carlo (MC) search was configured using the 'automatic setup' routine in MacroModel with all amide configurations in the macrocycle fixed as trans except for the amide bond between Ala-2 and Tyr-3. The calculation consisted of 50,000 MC steps with 500 iterations per step using the AMBER* force field and the PR conjugate gradient (PRCG) with no solvation.
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13. NMR data of analogue **3** in pyridine-d_5 at 300 K. Conformer-I: D-Ala-1 (α, δ_H 4.81/δ_C 50.4; β, δ_H 1.56 (3H)/δ_C 20.3; C=O, δ_C 173.1; NH, δ_H 8.11), Ala-2 (α, δ_H 4.76/δ_C 46.2; β, δ_H 1.47 (3H)/δ_C 16.6; C=O, δ_C 173.2; NH, δ_H 9.68), Tyr-3 (α, δ_H 4.15/δ_C 69.0; β, δ_H 3.88–3.94 (2H)/δ_C 33.7; γ, δ_C 131.9; δ, δ_H 7.27 (2H)/δ_C 130.9 (2C); ε, δ_H 7.00 (2H)/δ_C 114.4 (2C); ζ, δ_C 158.8; C=O, δ_C 169.5; NMe, δ_H 3.09 (3H)/δ_C 40.3; OMe, δ_H 3.70 (3H)/δ_C 55.2), Ala-4 (α, δ_H 5.28/δ_C 47.2; β, δ_H 1.46 (3H)/δ_C 18.6; C=O, δ_C 173.3; NH, δ_H 7.77), Tyr-5 (α, δ_H 5.00/δ_C 56.4; β, δ_H 2.61, 2.85/δ_C 36.7; γ, δ_C 138.5; δ_a, δ_H 7.30–7.38/δ_C 131.2; δ_b, δ_H 7.53/δ_C 133.1; ε_a, δ_H 7.30–7.38/δ_C 126.0; ε_b, δ_H 6.83/δ_C 126.0; ζ, δ_C 158.6; CH_2, δ_H 2.17, 2.35/δ_C 53.9; NMe, δ_H 2.76 (3H)/δ_C 28.8), Tyr-6 (α, δ_H 3.05/δ_C 67.8; β, δ_H 2.41, 3.50/δ_C 30.1; γ, δ_C 134.2; δ_a, δ_H 6.88/δ_C 121.9; δ_b, δ_H 4.73/δ_C 118.8; ε_a, δ_H 6.83/δ_C 113.0; ε_b, δ_C 154.0; ζ, δ_C 146.9; C=O, δ_C 171.5; NMe, δ_H 2.13 (3H)/δ_C 38.9; OMe, δ_H 3.84 (3H)/δ_C 56.3). Conformer-II: D-Ala-1 (α, δ_H 5.00/δ_C 50.9; β, δ_H 1.50 (3H)/δ_C 20.3; C=O, δ_C 173.1; NH, δ_H 8.66), Ala-2 (α, δ_H 5.15/δ_C 43.6; β, δ_H 0.96 (3H)/δ_C 17.8; C=O, δ_C 173.3; NH, δ_H 10.10), Tyr-3 (α, δ_H 5.78/δ_C 62.2; β, δ_H 3.21, 3.43/δ_C 34.2; γ, δ_C 130.1; δ, δ_H 7.26 (2H)/δ_C 130.8 (2C); ε, δ_H 6.94 (2H)/δ_C 114.6 (2C); ζ, δ_C 159.0; C=O, δ_C 168.2; NMe, δ_H 3.40 (3H)/δ_C 29.5; OMe, δ_H 3.64 (3H)/δ_C 55.2), Ala-4 (α, δ_H 5.22/δ_C 47.7; β, δ_H 1.26/δ_C 19.1; C=O, δ_C 172.9; NH, δ_H 9.13), Tyr-5 (α, δ_H 5.05/δ_C *; β, δ_H 2.85, */δ_C 36.2; γ, δ_C 138.6; δ_a, δ_H 7.30–7.38/δ_C 129.7; δ_b, δ_H 7.49/δ_C 133.2; ε_a, δ_H 7.30–7.38/δ_C 126.3; ε_b, δ_H 6.76/δ_C 126.1; ζ, δ_C 158.7; CH_2, δ_H */δ_C *; NMe, δ_H 2.81 (3H)/δ_C *), Tyr-6 (α, δ_H 3.09/δ_C 68.6; β, δ_H 2.32, 3.62/δ_C 29.7; γ, δ_C 134.5; δ_a, δ_H 6.98/δ_C 122.1; δ_b, δ_H 4.65/δ_C 119.0; ε_a, δ_H 6.87/δ_C 113.1; ε_b, δ_C 154.1; ζ, δ_C 146.9; C=O, δ_C 171.4; NMe, δ_H 2.05 (3H)/δ_C 39.4; OMe, δ_H 3.86 (3H)/δ_C 56.3). Remarks: * not assigned in the present experiment.
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