

## Asymmetric Synthesis of Double Bond Isomers of the Structure Proposed for Pyrinodemin A and Indication of Its Structural Revision

Haruaki Ishiyama, Masashi Tsuda, Tadashi Endo and Jun'ichi Kobayashi\*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan. Tel: (+81) 11-706-4985, Fax: (+81) 11-706-4989.

\* Author to whom correspondence should be addressed; E-mail: jkobay@pharm.hokudai.ac.jp

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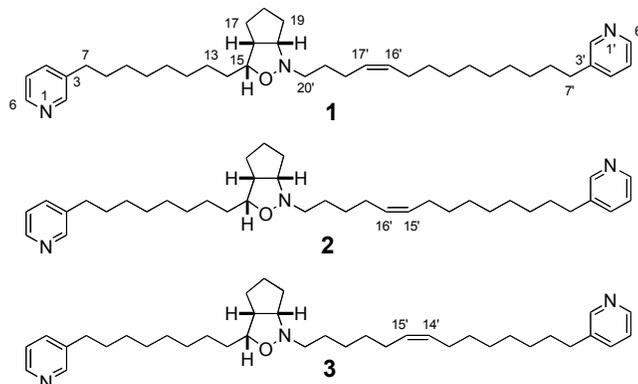
**Abstract:** Asymmetric synthesis of double bond isomers (+)-**2** ( $\Delta^{15',16'}$ ) and (+)-**3** ( $\Delta^{14',15'}$ ) of the structure **1** ( $\Delta^{16',17'}$ ) proposed for pyrinodemin A, a cytotoxic *bis*-pyridine alkaloid with a unique *cis*-cyclopent[*c*]isoxazolidine moiety from a marine sponge, has been accomplished. Pyrinodemin A was indicated to be a 1:1 racemic mixture of **2** from comparison of  $C_{18}$  and chiral HPLC analysis for pyrinodemin A and the synthetic compounds as well as ESIMS data of oxidative degradation products of pyrinodemin A.

**Keywords:** Pyrinodemin A, *Amphimedon* sp., asymmetric synthesis, structural revision

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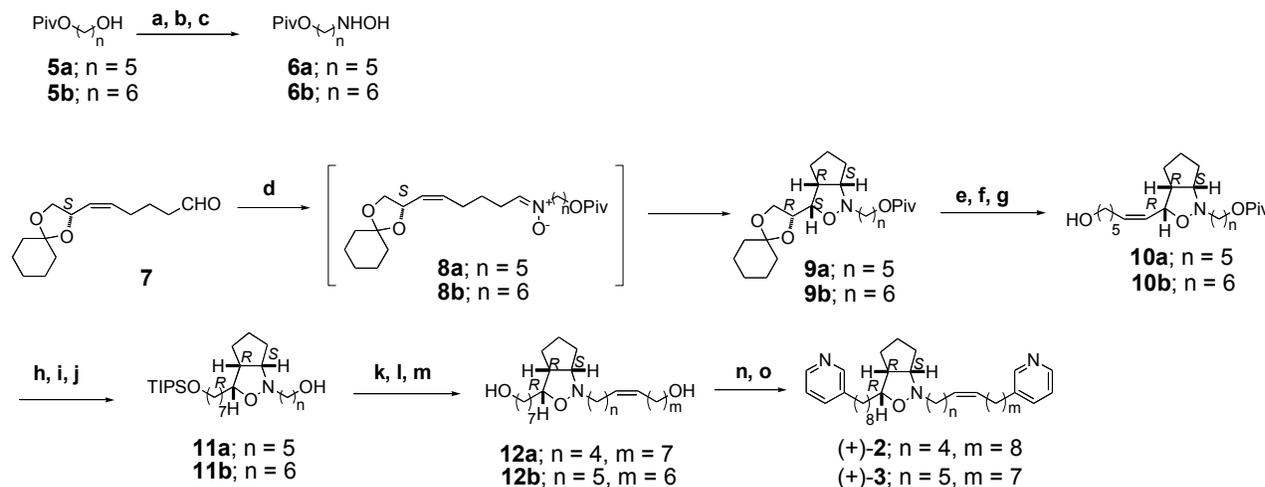
Pyrinodemin A, a cytotoxic *bis*-pyridine alkaloid with a unique *cis*-cyclopent[*c*]isoxazolidine moiety, has been isolated from a marine sponge *Amphimedon* sp., and its relative stereostructure was proposed as **1** ( $\Delta^{16',17'}$ ) on the basis of spectral data [1]. The unique structure of pyrinodemin A has prompted synthetic chemists to its total synthesis of **1** as well as syntheses of the double bond isomers **2** ( $\Delta^{15',16'}$ ) and **3** ( $\Delta^{14',15'}$ )[2-4] followed by different proposals of the structural revision of pyrinodemin A to be **2** [2] or **3** [3,4].

In order to examine the correct structure of pyrinodemin A, we have synthesized (+)-**2** and (+)-**3**, the double bond isomers of **1**, as an optically active form, and compared HPLC profiles of the synthetic compounds and pyrinodemin A. In addition, oxidative degradation experiments were performed for a remaining small amount of pyrinodemin A to determine the position of a double bond. In this paper, we describe asymmetric synthesis of (+)-**2** and (+)-**3**, and indication of the structure of pyrinodemin A to be ( $\pm$ )-**2**.



The  $\Delta^{15',16'}$  double bond isomer (+)-2 was synthesized as follows (Scheme 1). The synthesis of hydroxylamine **6a** commenced with known pivaloate **5a** [5]. Oxidation of alcohol **5a** with 2-iodobenzoic acid (IBX) [6] in DMSO and THF afforded its aldehyde. Treatment of the aldehyde with  $\text{NH}_2\text{OH}\cdot\text{HCl}$  and  $\text{NaOAc}$  in MeOH provided oxime which was reduced with  $\text{NaBH}_3\text{CN}$  in MeOH to afford hydroxylamine **6a** [7,8]. Condensation of **6a** and optically active aldehyde **7** [8] in  $\text{CHCl}_3$  containing  $\text{Na}_2\text{SO}_4$  at r.t. gave the nitron **8a**, which was followed by heating to afford *cis*-cyclopent[*c*]isoxazolidine [9] **9a** in 58% yield.

Scheme 1.



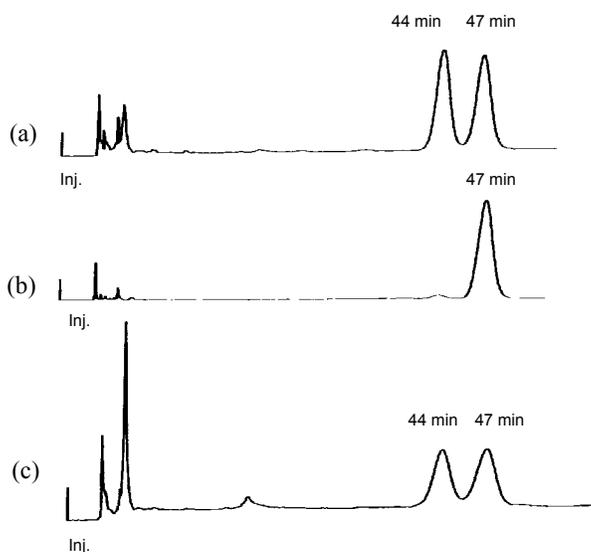
**Reagents and conditions:** (a) IBX, DMSO, THF (69%); (b)  $\text{H}_2\text{NOH}\cdot\text{HCl}$ ,  $\text{AcONa}$ , MeOH (96%); (c)  $\text{NaBH}_3\text{CN}$ , MeOH, pH 3, 0 °C; (d)  $\text{Na}_2\text{SO}_4$ , **6**,  $\text{CHCl}_3$ , r.t.~reflux (58% for 2 steps); (e) 3N HCl, dioxane (80%); (f)  $\text{NaIO}_4$ , MeCN,  $\text{H}_2\text{O}$ , 0 °C; (g)  $\text{Br}^-\text{[Ph}_3^+(\text{CH}_2)_5\text{CH}_2\text{OH]}$ , *n*-BuLi, THF, 0 °C (51% for 2 steps); (h) TIPSOTf, imidazole,  $\text{CH}_2\text{Cl}_2$  (75%); (i)  $\text{H}_2$ , Pd-C, MeOH (93%); (j) DIBAL,  $\text{CH}_2\text{Cl}_2$ , -78 °C (75%); (k) IBX, DMSO (80%); (l)  $\text{Br}^-\text{[Ph}_3^+(\text{CH}_2)_7\text{CH}_2\text{OH]}$ , *n*-BuLi, THF, 0 °C (81%); (m) 46% HF, MeCN (55%); (n)  $\text{CBr}_4$ ,  $\text{Ph}_3\text{P}$  (80%); (o) 3-methylpyridine, LDA, DMPU, -40 °C (64%)

Treatment of **9a** with 3N HCl in dioxane gave diol, which was converted into its aldehyde by treatment with  $\text{NaIO}_4$  and then into alcohol **10a** by Wittig reaction [10]. Protection of alcohol **10a** as its TIPS ether followed by reduction with Pd-C gave its saturated TIPS ether, which was converted

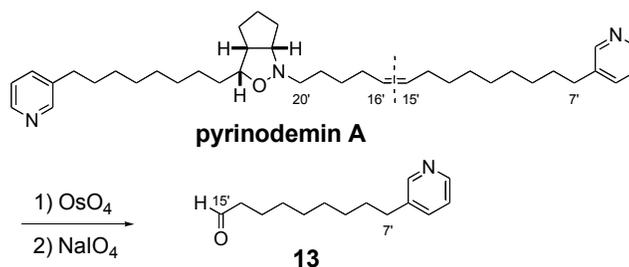
into alcohol **11a** with DIBAL. IBX oxidation of **11a** followed by Wittig reaction [10] afforded its unsaturated alcohol, which was subjected to deprotection with HF to give diol **12a** in 55 %. Treatment of diol **12a** with CBr<sub>4</sub> and PPh<sub>3</sub> provided its dibromide, which was coupled with 3-methylpyridine using LDA and DMPU [11] in THF to furnish optically active compound (+)-**2**. This is the first synthesis of optical active form of **2**, although its racemic form ((±)-**2**) has been synthesized [2-4]. The Δ<sup>14',15'</sup> double bond isomer (+)-**3** was prepared from pivaloate **5b** by almost same procedure as described for synthesis of (+)-**2** (Scheme 1).

The position of a double bond and the stereochemistry of pyrinodemin A were examined as follows. Compounds (±)-**1** [2], (±)-**2** [2], and (+)-**3** were subjected to C<sub>18</sub> HPLC [Wako sil-II 5C18 RS, Wako Ind., Ltd., 4.6 x 250 mm; flow rate 1.0 mL/min: eluent; MeOH/H<sub>2</sub>O (91:9); UV detection at 263 nm] and found to be separated (**1**, t<sub>R</sub> 21.6 min; **2**, t<sub>R</sub> 17.0 min; **3**, t<sub>R</sub> 15.8 min), while the retention time (t<sub>R</sub> 17.0 min) of pyrinodemin A was identical with that of **2** under the same condition, indicating that the position of a double bond of pyrinodemin A corresponded to that (Δ<sup>15',16'</sup>) of **2**. To elucidate the stereochemistry of pyrinodemin A, compound (±)-**2** was subjected to chiral HPLC [CHIRALCELL OD-H, Daicel Co., Ltd., 4.6 x 250 mm; flow rate 1.0 mL/min: eluent: hexanes/*i*-PrOH (95:5); UV detection at 263 nm] and found to be separated (t<sub>R</sub> 44 and 47 min), while the retention time of (+)-**2** was 47 min (Figure 1). On the other hand, pyrinodemin A gave the two peaks corresponding to those of (±)-**2** in a ratio of 1:1 under the same conditions, indicating that pyrinodemin A is a 1:1 racemic mixture of **2**. Furthermore, pyrinodemin A was treated with OsO<sub>4</sub> and then NaIO<sub>4</sub> to give degradation products, one of which showed an ESIMS fragment ion peak at *m/z* 242 (M+Na)<sup>+</sup>, corresponding to an aldehyde (**13**) of C-7'~C-15' segment connected to a pyridine ring (Scheme 2). From the results described above, it was indicated that the olefin position of pyrinodemin A was C-15' and C-16' (**2**), as proposed by Snider's group [2], and that pyrinodemin A was a 1:1 racemic mixture of **2**.

**Figure 1.** Chiral HPLC profiles of (a) synthetic compounds (±)-**2**, (b) (+)-**2**, and (c) pyrinodemin A



## Scheme 2



## Acknowledgments

We thank Professor B. B. Snider (Brandeis University) for generous offer of synthetic samples of (±)-**1** and (±)-**2**. This work was supported in part by grants from the Akiyama Foundation and the Takeda Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

## Experimental

## General

Optical rotations were determined on a JASCO P-1030 polarimeter. Infrared spectra were obtained on a JASCO FT/IR-230 spectrometer. Proton and carbon NMR spectra were recorded on a Bruker 600 MHz spectrometer. Chemical shifts are reported in  $\delta$  values relative to chloroform ( $\delta$  7.26 for proton and  $\delta$  77.0 for carbon NMR). EI mass spectra were measured on a JEOL JMS-DX303 spectrometer.

*Synthetic Compound (+)-2*:  $[\alpha]^{25}_D +5.5^\circ$  ( $c$  0.6, CHCl<sub>3</sub>); IR (neat) 1575 cm<sup>-1</sup>; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  1.25~1.50 (26H, m), 1.50~1.74 (9H, m), 1.75 (1H, m), 2.01 (4H, m), 2.60 (5H, m), 2.91 (2H, m), 3.50 (1H, m), 4.15 (1H, m), 5.33 (2H, m), 7.22 (2H, m), 7.51 (2H, m), 8.44 (4H, m); <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  26.3, 26.4, 27.0, 27.1, 27.2, 27.5, 27.8, 29.1, 29.3, 29.4, 29.7, 31.1, 33.0, 34.2, 49.9, 57.1, 72.6, 77.7, 123.2, 129.6, 130.0, 135.7, 137.9, 147.1, 149.9; HREIMS  $m/z$  573.4643 [M<sup>+</sup>; calcd for C<sub>38</sub>H<sub>59</sub>N<sub>3</sub>O<sub>1</sub> 573.4658].

*Synthetic Compound (+)-3*:  $[\alpha]^{25}_D +6.2^\circ$  ( $c$  0.8, CHCl<sub>3</sub>); IR (neat) 1575 cm<sup>-1</sup>; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  1.25~1.50 (26H, m), 1.50~1.74 (9H, m), 1.77 (1H, m), 2.00 (4H, m), 2.58 (5H, m), 2.82 (2H, m), 3.45 (1H, m), 4.04 (1H, m), 5.33 (2H, m), 7.18 (2H, m), 7.47 (2H, m), 8.43 (4H, m); <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  26.3, 26.4, 27.1, 28.0, 28.8, 29.1, 31.1, 33.0, 34.3, 49.9, 57.3, 77.7, 123.2, 129.8, 135.7, 137.9, 147.1, 149.9; HREIMS  $m/z$  573.4661 [M<sup>+</sup>; calcd for C<sub>38</sub>H<sub>59</sub>N<sub>3</sub>O<sub>1</sub> 573.4658].

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*Sample Availability:* Available from the authors.