

# Synthesis of the CDE/FG Ring Models of Prymnesins: Reassignment of the Relative Configuration of the E/F Ring Juncture

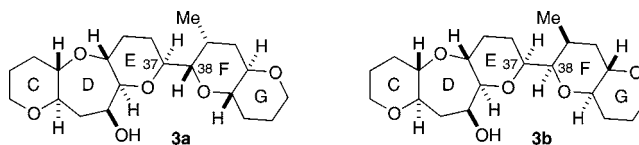
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Received March 8, 2004

## ABSTRACT



Synthesis of two diastereomeric models (3a and 3b) corresponding to the CDE/FG ring of prymnesins, polycyclic ether toxins isolated from the red tide phytoflagellate *Prymnesium parvum*, is described. Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data for each compound with those reported for prymnesins suggests that the earlier stereochemical assignment of the E/F ring juncture needs to be revised.

*Prymnesium parvum* is a unicellular alga that blooms in brackish water and causes massive fish kills worldwide. The causative toxins, prymnesin-1 (PRM1, **1**) and prymnesin-2 (PRM2, **2**), were isolated from cultured cells of the phytoflagellate. These toxins possess extremely potent hemolytic activity, which is about 5000-fold greater than that of Merk saponin on a molar basis, and also exhibit potent ichthyotoxicity. Their gross structures, including partial stereochemistry, were determined by Igarashi et al.<sup>1,2</sup> Prymnesins possess unique structural features: an unbranched single chain of 90 carbons except for a single methyl group, a fused polycyclic ether ring system (A–E ring), four distinct 1,6-dioxadecalin units (FG, HI, JK, and

LM rings), conjugated double and triple bonds, chlorine atoms and an amino group, and glycosidic residues, including an uncommon L-xylose. The relative stereochemistry of the fused A–E polycyclic ether ring domain and four 1,6-dioxadecalin units was determined by extensive NMR analysis. Recently, the absolute configuration at C14 bearing an amino group in PRM2 was determined to be *S* by using a chiral anisotropic reagent and that at chlorinated C85 to be *S* by fluorimetric chiral HPLC comparison between a degradation product and synthetic references (Figure 1).<sup>3</sup>

On the basis of the extensive NOE data as well as coupling constants, it was proposed that the E/F ring juncture adopted a twisted gauche rotamer, where two pairs of diaxial protons (33-H/37-H and 38-H/42-H) were aligned under approximately 20° of the dihedral angle.<sup>2</sup> Although this conformation best explained the observed NMR data, it remained some-

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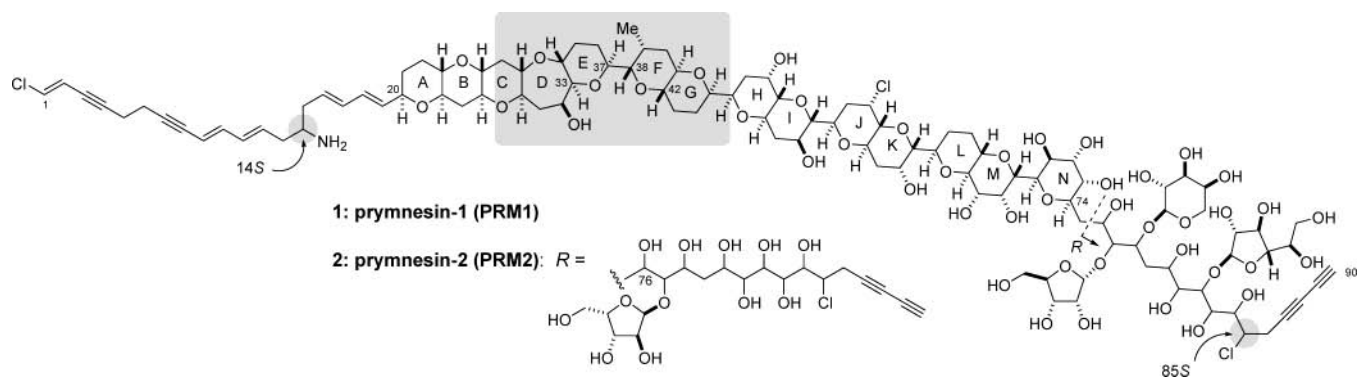
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(1) Igarashi, T.; Satake, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1996**, *118*, 479–480.

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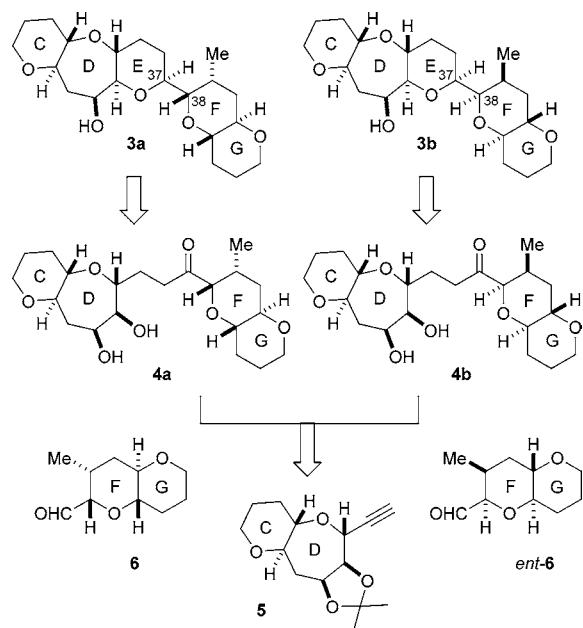
**Figure 1.** Proposed structures of prymnesin-1 (PRM1, **1**) and prymnesin-2 (PRM2, **2**).

what ambiguous for the relative configuration between the C-37 and C-38 positions.

We have already demonstrated that the synthesis of model fragments coupled with *J*-based configuration analysis (JBCA)<sup>4</sup> has successfully elucidated the relative configuration of the acyclic portions of maitotoxin, the most toxic and largest nonbiopolymer.<sup>5</sup> A similar approach was applied to prymnesins, and the relative configuration of the *I/J* ring juncture was unambiguously confirmed.<sup>6</sup> As part of our studies toward complete stereochemical assignment of prymnesins, we describe herein the synthesis of two diastereomeric CDE/FG ring models **3a** and **3b** for comparison of their NMR data with those of prymnesins, which led to reassignment of the proposed stereochemical assignment of the E/F ring juncture of the natural products.

Retrosynthetic analysis for convergent synthesis of model compounds **3a** and **3b** is outlined in Scheme 1. We

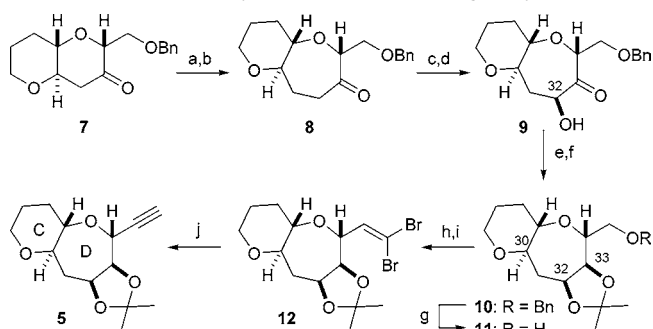
**Scheme 1.** Retrosynthetic Analysis



envisioned that the six-membered E ring within **3a** and **3b** could be formed by reductive cyclization of dihydroxy ketones **4a** and **4b**. These intermediates, in turn, would be obtained through convergent coupling of the acetylide anion generated from alkyne **5** with aldehydes **6** and *ent*-**6**.

The synthesis of the CD ring alkyne **5** started with bicyclic ketone **7** (Scheme 2). Ring expansion<sup>8</sup> of **7** with tri-

**Scheme 2.** Synthesis of the CD Ring Alkyne<sup>a</sup>



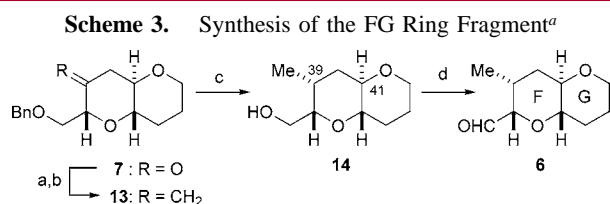
<sup>a</sup> Reagents and conditions: (a) trimethylsilyldiazomethane,  $\text{BF}_3 \cdot \text{OEt}_2$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ ; (b) CSA, MeOH, rt, 67% (two steps); (c) LiHMDS,  $\text{Et}_3\text{N}$ , TMSCl, THF,  $-78^\circ\text{C}$ ; (d)  $\text{OsO}_4$  (cat.), NMO, THF/ $\text{H}_2\text{O}$ , rt, 89% (two steps); (e) DIBAL-H,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ ; (f)  $\text{Me}_2\text{C}(\text{OMe})_2$ , CSA,  $\text{CH}_2\text{Cl}_2$ , rt, 95% (two steps); (g)  $\text{H}_2$ ,  $\text{Pd}(\text{OH})_2/\text{C}$ , MeOH, rt, 97%; (h)  $\text{SO}_3 \cdot \text{pyr}$ ,  $\text{Et}_3\text{N}$ , DMSO,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ ; (i)  $\text{CBr}_4$ ,  $\text{PPh}_3$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 85% (two steps); (j) NaHMDS, THF,  $-100^\circ\text{C}$ , then *n*-BuLi, 90%.

methylsilyldiazomethane in the presence of  $\text{BF}_3 \cdot \text{OEt}_2$  gave, after acidic treatment, seven-membered ketone **8** in 67% yield for the two steps. Ketone **8** was converted to the corresponding silyl enol ether, which was then oxidized with  $\text{OsO}_4$  to afford  $\alpha$ -hydroxy ketone **9** in 89% yield as the sole product.<sup>9</sup> DIBAL-H reduction of **9** followed by protection of the resultant diol gave acetonide **10** in 95% overall yield. The stereochemistry of **10** was unambiguously established by

(4) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.

NOEs between 30-H/32-H and 32-H/33-H.<sup>10</sup> Removal of the benzyl group by hydrogenolysis provided alcohol **11** (97%), which was then converted into dibromoolefin **12** in two steps (85% yield).<sup>11</sup> Finally, sequential treatment with NaHMDS and *n*-BuLi afforded the desired alkyne **5** in 90% yield.<sup>12</sup>

For the construction of the FG ring aldehyde **6**, we used ketone **7** as the same starting material, which was converted to *exo*-methylene **13** through Peterson olefination<sup>13</sup> (Scheme 3). This olefin was subjected to hydrogenation/hydrogenoly-

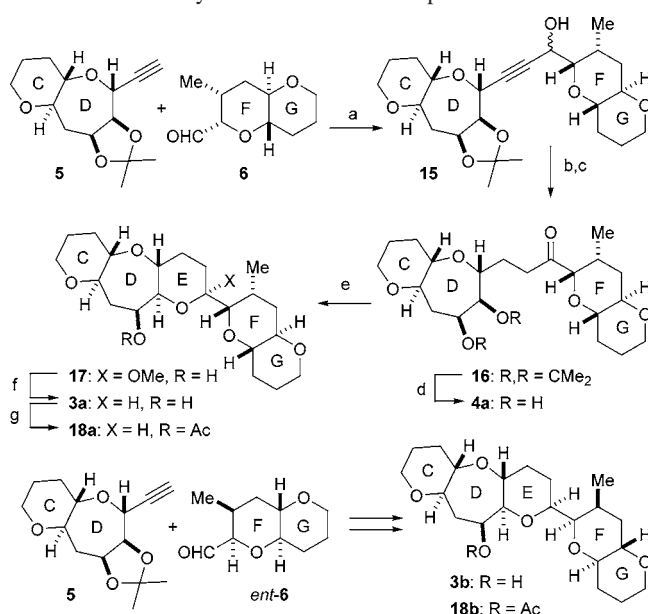


<sup>a</sup> Reagents and conditions: (a) TMSCH<sub>2</sub>MgCl, THF, 0 °C; (b) HOAc, 120 °C, 55% (two steps); (c) H<sub>2</sub>, Pd/C, EtOAc, rt, 50%; (d) SO<sub>3</sub>·pyr, Et<sub>3</sub>N, DMSO, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 85%.

sis to produce an approximately 2:1 mixture of reduced products, from which the desired alcohol **14** was separated by column chromatography on silica gel in 50% yield.<sup>14</sup> The stereochemistry of the axial-oriented methyl group at C39 was confirmed by NOE between 39-Me and 41-H.<sup>10</sup> Oxidation of **14** gave aldehyde **6** in 85% yield.

With the desired **5** and **6** in hand, we next focused our attention on their coupling (Scheme 4). An initial attempt to couple the lithium acetylide generated from **5** (*n*-BuLi, THF, -78 °C) with **6** gave a poor yield of the desired propargylic

**Scheme 4.** Synthesis of Model Compounds **3a** and **3b**<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) **5**, *n*-BuLi, THF, -78 °C, CeCl<sub>3</sub>, then **6**, 93%; (b) H<sub>2</sub>, Pd/C, MeOH, rt; (c) TPAP, NMO, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, rt, 80% (two steps); (d) 6 M HCl, THF, 0 → 30 °C, 89%; (e) *p*-TsOH·H<sub>2</sub>O, MeOH, rt, 44% (92% based on recovered **4a**); (f) Et<sub>3</sub>SiH, BF<sub>3</sub>·OEt<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/MeCN, 0 °C, 83%; (g) Ac<sub>2</sub>O, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt, quant.

alcohol **15** together with recovered **5** and **6**. However, a less basic alkynylcerium reagent<sup>15</sup> prepared from the lithium acetylide and cerium(III) chloride was more satisfactory and added successfully to aldehyde **6** to give **15** as an approximately 7:3 diastereomeric mixture in high yield. Hydrogenation of the triple bond followed by oxidation of the secondary alcohol with TPAP/NMO provided ketone **16** in 80% yield over the two steps. The acetonide group was removed by treatment with 6 M HCl (89%), and the obtained keto diol **4a** was then treated with *p*-toluenesulfonic acid in methanol to yield methyl ketal **17** in 44% yield along with recovered **4a** (52%).<sup>16</sup> Finally, reduction of **17** with triethylsilane in the presence of BF<sub>3</sub>·OEt<sub>2</sub> furnished the CDE/FG ring model **3a** in 83% yield.<sup>17</sup> The stereochemistry of **3a** was unambiguously established by NOE and coupling constant data. Similarly, the diastereomeric model **3b** was prepared from alkyne **5** and aldehyde *ent*-**6**.<sup>18</sup>

The two diastereomeric CDE/FG ring models **3a** and **3b** were subjected to the NMR study, and the <sup>1</sup>H and <sup>13</sup>C NMR

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(10) Numbering of all compounds in this paper corresponds to that of pyrimnesins.

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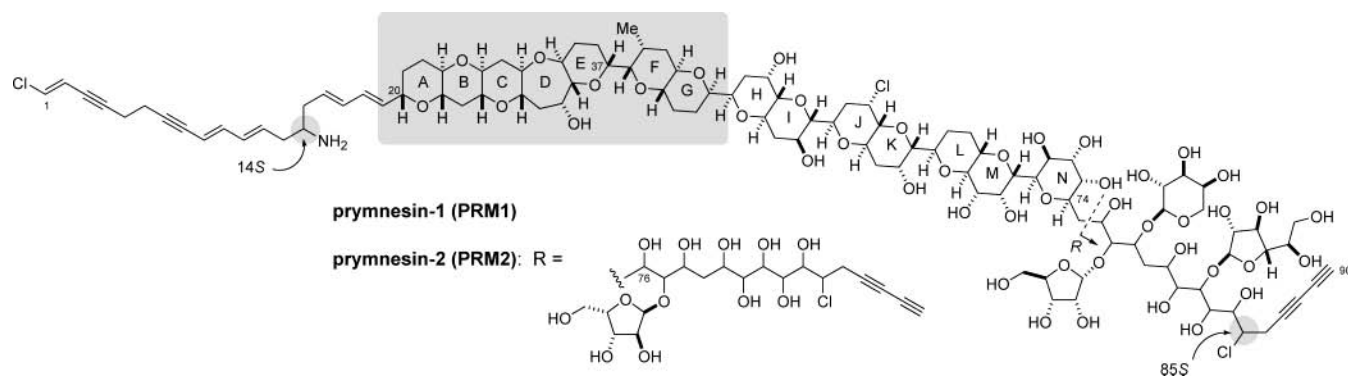
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(16) Direct treatment of **16** with excess amounts of *p*-toluenesulfonic acid in methanol resulted in a low yield of methyl ketal **17**.

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(18) Synthesis of compounds *ent*-**6** and **3b** are included in Supporting Information.



**Figure 2.** Revised structure of prymnesins.

chemical shifts for the C35–C40 portion of each compound were compared with those of *N*-acetylprymnesin-2 (NAPRM2) (Table 1). The NMR data observed for **3b**, and not for **3a**,

**Table 1.** Selected NMR Data of the C35–C40 Regions in NAPRM2 and Model Compounds **3a** and **3b** (600 MHz, 1:1 C<sub>5</sub>D<sub>5</sub>N/CD<sub>3</sub>OD)

	NAPRM2		<b>3a</b>		<b>3b</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
35	1.37	32.4	1.42	32.4 <sup>a</sup>	1.38	32.3
	2.02		2.03		2.03	
36	1.23	30.9	1.22	28.4	1.24	30.6
	1.94		1.52		1.96	
37	3.07	77.9	3.24	79.7	3.09	77.6
38	3.12	84.4	3.31	84.4	3.16	83.9
39	2.04	31.6	1.81	32.5 <sup>a</sup>	2.09	31.3
40	1.44	38.5	1.52	39.0	1.45	38.1
	1.67		1.71		1.69	
39-Me	0.88	14.5	0.89	14.5	0.91	14.2

<sup>a</sup> Interchangeable.

matched well the reported values for NAPRM2. In particular, the  $\delta_{\text{C}}$  values of C36 and C37 in **3a** deviated from those of NAPRM2 over 1 ppm, while the relevant values of **3b** match those of NAPRM2 within 0.5 ppm. The coupling constants,  $J_{37,38} = 9.0$  Hz and  $J_{38,39} = 2.5$  Hz, of **3b** also agreed well with those reported for NAPRM2 (8.5 and 2.5 Hz, respectively), while the diastereomer **3a** showed somewhat different values (7.8 and 1.9 Hz, respectively). In addition, NMR chemical shift values for acetate **18b** (600 MHz, CDCl<sub>3</sub>) corresponded well with those for peracetyl prymnesin-2 (PAPRM2), while those for acetate **18a** did not (Table 2). These results suggest that the formerly assigned configuration

of the E/F ring juncture needs to be revised to be represented by structure **3b**, though the NOE data of **3b** did not fully reproduce those observed for NAPRM2.<sup>19</sup>

**Table 2.** Selected NMR Data of the C35–C40 Regions in PAPRM2 and Model Compounds **18a** and **18b** (600 MHz, CDCl<sub>3</sub>)

	PAPRM2		<b>18a</b>		<b>18b</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
35	1.46	30.8	1.44	30.9	1.45	30.8
	2.11		2.10		2.08	
36	1.27	29.1	1.24	26.5	1.26	29.1
	2.05		1.64		2.04	
37	3.11	76.4	3.22	78.1	3.10	76.2
38	3.23	82.1	3.32	82.3	3.24	82.1
39	2.17	29.5	1.92	30.4	2.17	29.5
40	1.53	36.4	1.58	37.3	1.55	36.5
	1.78		1.80		1.79	
39-Me	0.93	13.0	0.96	13.2	0.94	13.0

In conclusion, we have synthesized two diastereomeric CDE/FG ring models **3a** and **3b** of prymnesins through alkynylcerium–aldehyde coupling and reductive ring-closure of the E ring. Comparison of the NMR chemical shifts for models **3a** and **3b** with those reported for natural products allowed the reassignment of the relative configuration of the E/F ring juncture of prymnesins to be that shown in Figure 2.

**Acknowledgment.** We are grateful to Professor Kazuo Tachibana (University of Tokyo) for helpful discussion. This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**Supporting Information Available:** Experimental procedure and spectroscopic data for all compounds; synthetic scheme and NOE data for compound **3b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(19) NOE data for compound **3b** is included in Supporting Information.