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

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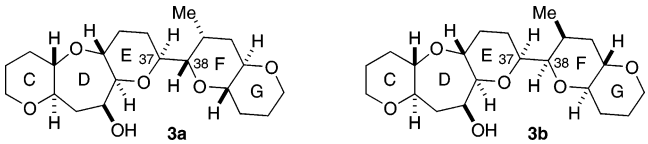
ABSTRACT



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 ^{1}H and ^{13}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.[1,2] 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).[3]

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.[2] Although this conformation best explained the observed NMR data, it remained some-

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(1) Igarashi, T.; Satake, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1996**, *118*, 479−480.
(2) Igarashi, T.; Satake, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1999**, *121*, 8499−8511.
(3) Morohashi, A.; Satake, M.; Oshima, Y.; Igarashi, T.; Yasumoto, T. *Chirality* **2001**, *13*, 601−605.

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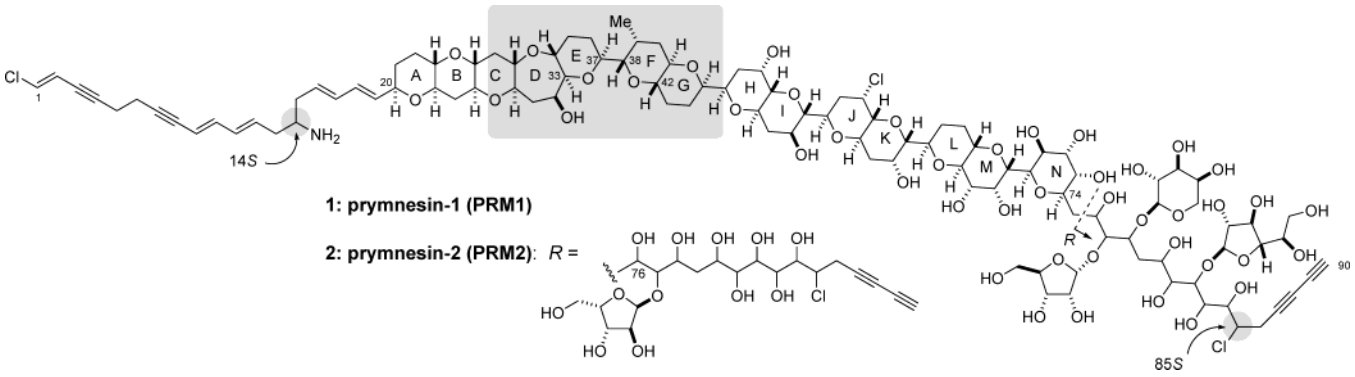


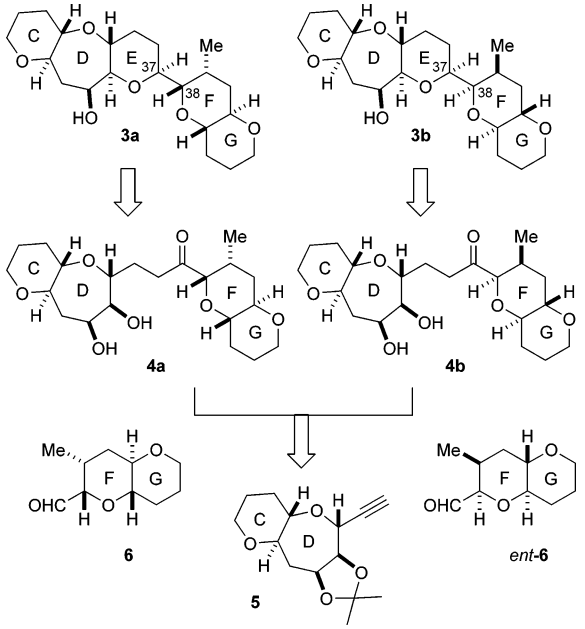
Figure 1. Proposed structures of prymnesin-1 (PRM1, **1**) and prymnesin-2 (PRM2, **2**).

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)[4] has successfully elucidated the relative configuration of the acyclic portions of maitotoxin, the most toxic and largest nonbiopolymer.[5] A similar approach was applied to prymnesins, and the relative configuration of the I/J ring juncture was unambiguously confirmed.[6] 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.

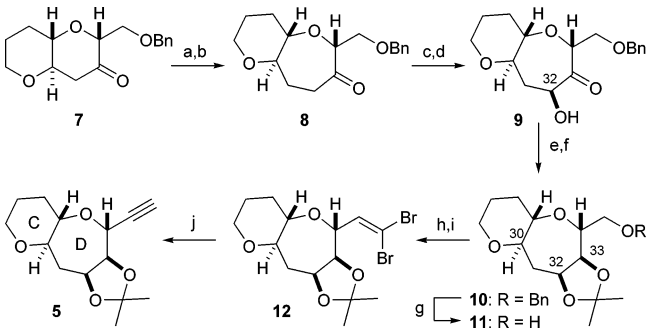
Retrosynthetic analysis for convergent synthesis of model compounds **3a** and **3b** is outlined in Scheme 1. We envisioned that the six-membered E ring within **3a** and **3b** could be formed by reductive cyclization of dihydroxy ketones **4a** and **4b**. These intermediates, in turn, would be obtained through convergent coupling of the acetylide anion generated from alkyne **5** with aldehydes **6** and *ent*-**6**.

Scheme 1. Retrosynthetic Analysis



The synthesis of the CD ring alkyne **5** started with bicyclic ketone **7**[7] (Scheme 2). Ring expansion[8] of **7** with trimethylsilyldiazomethane in the presence of $BF_3{\cdot}OEt_2$ 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 OsO_4 to afford α-hydroxy ketone **9** in 89% yield as the sole product.[9] 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

Scheme 2. Synthesis of the CD Ring Alkyne[a]



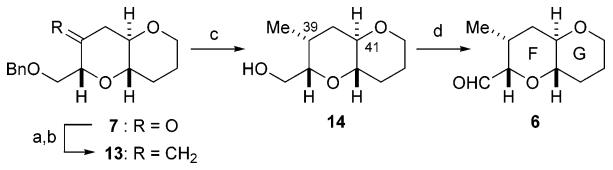
[a] Reagents and conditions: (a) trimethylsilyldiazomethane, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C; (b) CSA, MeOH, rt, 67% (two steps); (c) LiHMDS, Et_3N, TMSCl, THF, −78 °C; (d) OsO_4 (cat.), NMO, THF/H_2O, rt, 89% (two steps); (e) DIBAL-H, CH_2Cl_2, −78 °C; (f) $Me_2C(OMe)_2$, CSA, CH_2Cl_2, rt, 95% (two steps); (g) H_2, $Pd(OH)_2$/C, MeOH, rt, 97%; (h) $SO_3{\cdot}pyr$, Et_3N, DMSO, CH_2Cl_2, 0 °C; (i) CBr_4, PPh_3, CH_2Cl_2, rt, 85% (two steps); (j) NaHMDS, THF, −100 °C, then *n*-BuLi, 90%.

(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.[10] Removal of the benzyl group by hydrogenolysis provided alcohol **11** (97%), which was then converted into dibromoolefin **12** in two steps (85% yield).[11] Finally, sequential treatment with NaHMDS and *n*-BuLi afforded the desired alkyne **5** in 90% yield.[12]

For the construction of the FG ring aldehyde **6**, we used ketone **7**[7] as the same starting material, which was converted to *exo*-methylene **13** through Peterson olefination[13] (Scheme 3). This olefin was subjected to hydrogenation/hydrogenolysis 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.[14] The stereochemistry of the axial-oriented methyl group at C39 was confirmed by NOE between 39-Me and 41-H.[10] Oxidation of **14** gave aldehyde **6** in 85% yield.

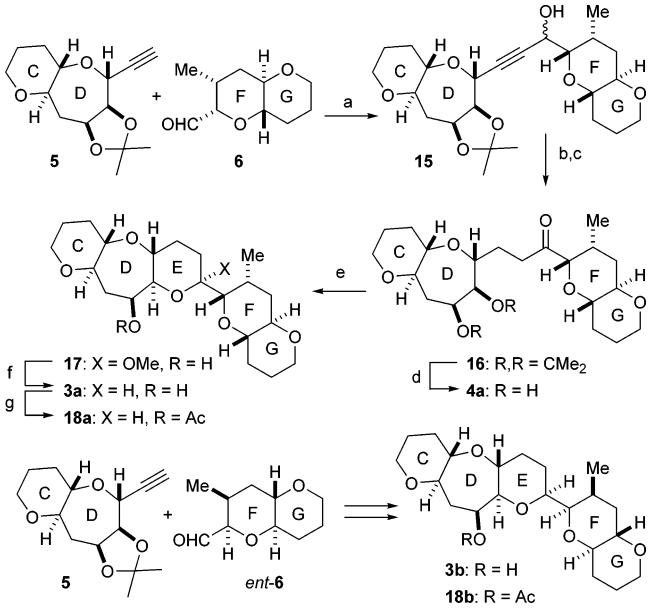
Scheme 3. Synthesis of the FG Ring Fragment[a]



[a] Reagents and conditions: (a) $TMSCH_2MgCl$, THF, 0 °C; (b) HOAc, 120 °C, 55% (two steps); (c) H_2, Pd/C, EtOAc, rt, 50%; (d) SO_3·pyr, Et_3N, DMSO, CH_2Cl_2, 0 °C, 85%.

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 alcohol **15** together with recovered **5** and **6**. However, a less basic alkynylcerium reagent[15] 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%).[16] Finally, reduction of **17** with triethylsilane in the presence of BF_3·OEt_2 furnished the CDE/FG ring model **3a** in 83% yield.[17] 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**.[18]

Scheme 4. Synthesis of Model Compounds **3a** and **3b**[a]



[a] Reagents and conditions: (a) **5**, *n*-BuLi, THF, −78 °C, $CeCl_3$, then **6**, 93%; (b) H_2, Pd/C, MeOH, rt; (c) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt, 80% (two steps); (d) 6 M HCl, THF, 0 → 30 °C, 89%; (e) *p*-TsOH·H_2O, MeOH, rt, 44% (92% based on recovered **4a**); (f) Et_3SiH, BF_3·OEt_2, CH_2Cl_2/MeCN, 0 °C, 83%; (g) Ac_2O, DMAP, CH_2Cl_2, rt, quant.

(5) (a) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1994**, *35*, 5023−5026. (b) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 9007−9010. (c) Sasaki, M.; Matsumori, N.; Murata, M.; Tachibana, K.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 9011−9014. (d) Sasaki, M.; Matsumori, N.; Maruyama, T.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1782−1785. (e) Nonomura, T.; Sasaki, M.; Matsumori, N.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1786−1789. For the work from Kishi's group, see: (f) Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946−7968.

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(9) For similar stereoselective introduction of a hydroxy group into a seven-membered ether, see: Tsukano, C.; Sasaki, M. *J. Am. Chem. Soc.* **2003**, *125*, 14294−14295.

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(15) (a) Imamoto, T.; Kusumoto, T.; Tawarayama, Y.; Sugiura, Y.; Mita, T.; Hatanaka, Y.; Yokoyama, M. *J. Org. Chem.* **1984**, *49*, 3904−3912. (b) Imamoto, T.; Sugiura, Y.; Takiyama, N. *Tetrahedron Lett.* **1984**, *25*, 4233−4236. (c) Fox, C. M. J.; Hiner, R. N.; Warrier, U.; White, J. D. *Tetrahedron Lett.* **1988**, *29*, 2923−2926. (d) Molander, G. A. *Chem. Rev.* **1992**, *92*, 29−68.

(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.

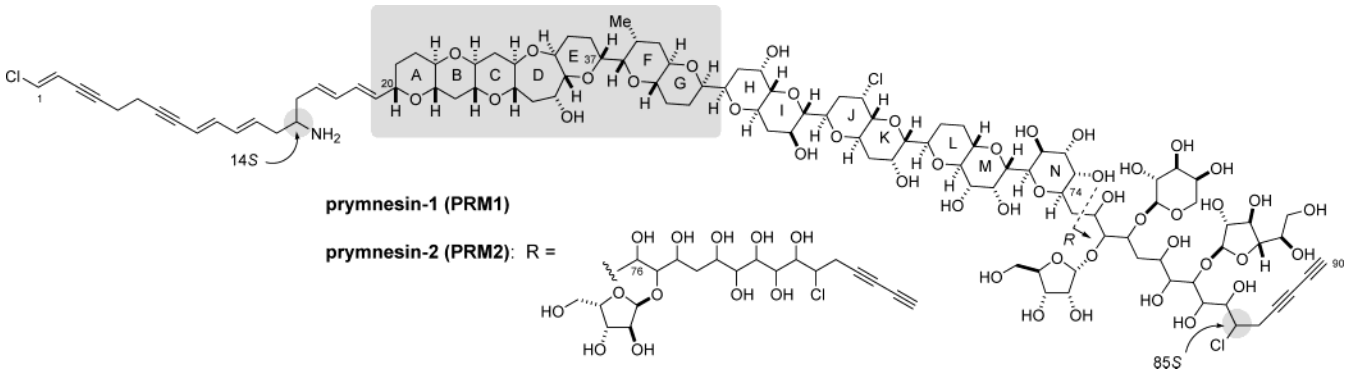

Figure 2. Revised structure of prymnesins.

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**, matched well the reported values for NAPRM2. In particular, the δ_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, $CDCl_3$) 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.[19]

Table 1. Selected NMR Data of the C35−C40 Regions in NAPRM2 and Model Compounds **3a** and **3b** (600 MHz, 1:1 C_5D_5N/CD_3OD)

	NAPRM2		**3a**		**3b**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
35	1.37	32.4	1.42	32.4[a]	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[a]	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

[a] Interchangeable.

Table 2. Selected NMR Data of the C35−C40 Regions in PAPRM2 and Model Compounds **18a** and **18b** (600 MHz, $CDCl_3$)

	PAPRM2		**18a**		**18b**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_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 synthesiszed 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.

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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.