Pergamon

Tetrahedron Letters 42 (2001) 5725–5728

TETRAHEDRON LETTERS

Synthesis and stereochemical confirmation of the HI/JK ring system of prymnesins, potent hemolytic and ichthyotoxic glycoside toxins isolated from the red tide alga

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Received 28 May 2001; revised 14 June 2001; accepted 15 June 2001

Abstract—Stereocontrolled synthesis of the HI/JK ring model of the prymnesins, glyosidic toxins isolated from the red tide phytoflagellate *Prymnesium parvum*, is described. Comparison of its ^{1}H and ^{13}C NMR data with those of the natural toxins established the earlier stereochemical assignments.

Two glycosidic toxins, prymnesin-1 (PRM1, **1**) and prymnesin-2 (PRM2, **2**), were isolated from cultured cells of the red tide phytoflagellate *Prymnesium parvum*.[1] These toxins possess extremely potent hemolytic activity, which is about 5,000-fold greater than that of Merk saponin on a molar basis and also exhibit potent ichthyotoxicity.[2] Their gross structures and partial stereochemical assignments have been disclosed by Igarashi and Yasumoto.[3,4] Prymnesins possess unique structural features: an unbranched single chain of 90 carbons except for a single methyl group, a fused polyether ring system (A–E ring), four distinct 1,6-dioxadecalin units, conjugated double and triple bonds, chlorine and nitrogen atoms, and an uncommon L-xylose. The relative stereochemistry of the fused A–E polyether ring domain and four 1,6–dioxadecalin units (rings FG, HI, JK, and LM) was determined by extensive NMR analysis. The diastereomeric relationship among these polyethers was assigned by the extensive NOE and $^{3}J_{H,H}$ analysis, indicating that all ring linkages, except for that of rings I/J, took the C–C *anti* conformation with respect to the C–C linking bond. For rings I/J, an exceptional C–C *gauche* rotamer was proposed on the basis of NOE analysis; however, force field calculations suggested a C–C *anti* system as the lowest energy rotamer.[5] Accordingly, we decided to synthesize the HI/JK ring model to confirm the assigned stereochemistry. We have already demonstrated the utility of a synthetic approach for the configurational assignment of acyclic portions of maitotoxin, the most toxic and largest non-biopolymer.[6] As part of our studies toward complete stereochemical assignment of prymnesins, we describe herein the stereoselective synthesis of the HI/JK ring model **3**, which culminated in the confirmation of the proposed stereochemical assignment.

Synthesis of the H ring alkyne **9** started with tri-*O*-acetyl-D-glucal (**4**), which was converted to alcohol **5** in three steps (Scheme 1). An oxidation–reduction sequence allowed inversion of the hydroxyl group to give **6** in 78% yield. Benzylation followed by removal of the acetonide group provided diol **7**, which upon regioselective activation and protection by a one-pot procedure[7] gave triflate **8**. Alkylation of **8** with lithium (trimethylsilyl)acetylide was followed by desilylation to give the desired alkyne **9** in 64% overall yield.

Construction of the JK ring lactone **15** is shown in Scheme 2. Routine protective group manipulation allowed conversion of the known alcohol **10**[8] into alcohol **11**. Inversion of the secondary alcohol followed by deprotection of the primary alcohol and benzylation gave bis-benzyl ether **12** in 98% overall yield. Oxidative cleavage of the double bond provided the corresponding aldehyde, which upon chelation-controlled allylation with allyltributyltin and $MgBr_2 \cdot OEt_2$[9] yielded

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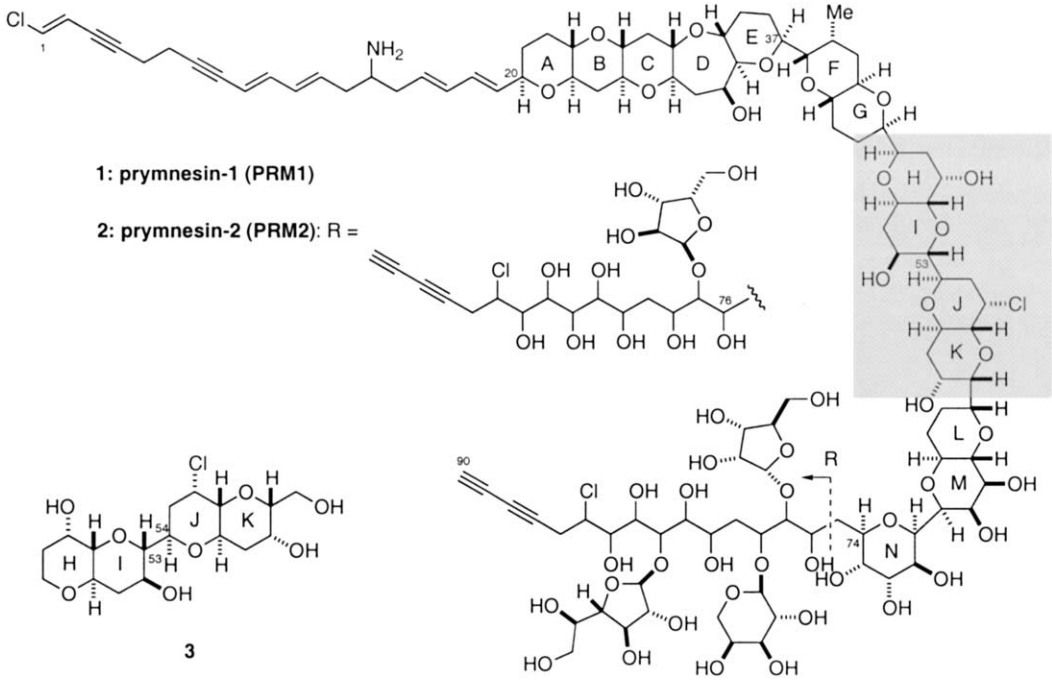
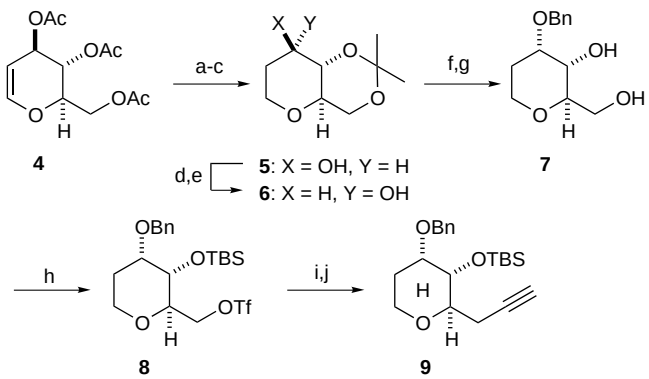
0040-4039/01/$ - see front matter
PII: S0040-4039(01)01067-X



homoallylic alcohol **13** in 90% yield along with the undesired diastereomer **14** (7% yield). Protection as its *p*-methoxybenzyl (MPM) ether, desilylation, oxidative cleavage of the terminal olefin and oxidation of the derived hemiacetal with TPAP/NMO[10] furnished the desired lactone **15** in 84% overall yield.

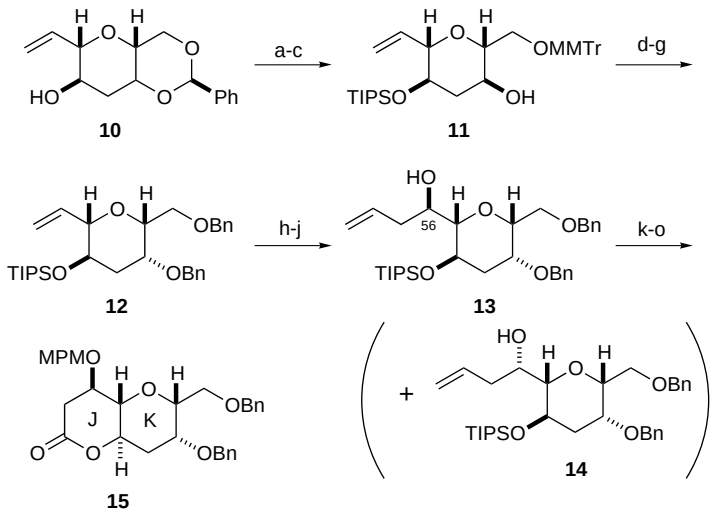
Lithium acetylide generated from **9** was reacted with lactone **15** in THF–HMPA to produce hemiketal **16**. Axial hydride reduction of the C54[11] hemiketal (Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −30°C) afforded alkyne **17** in 68% overall yield as a single stereoisomer



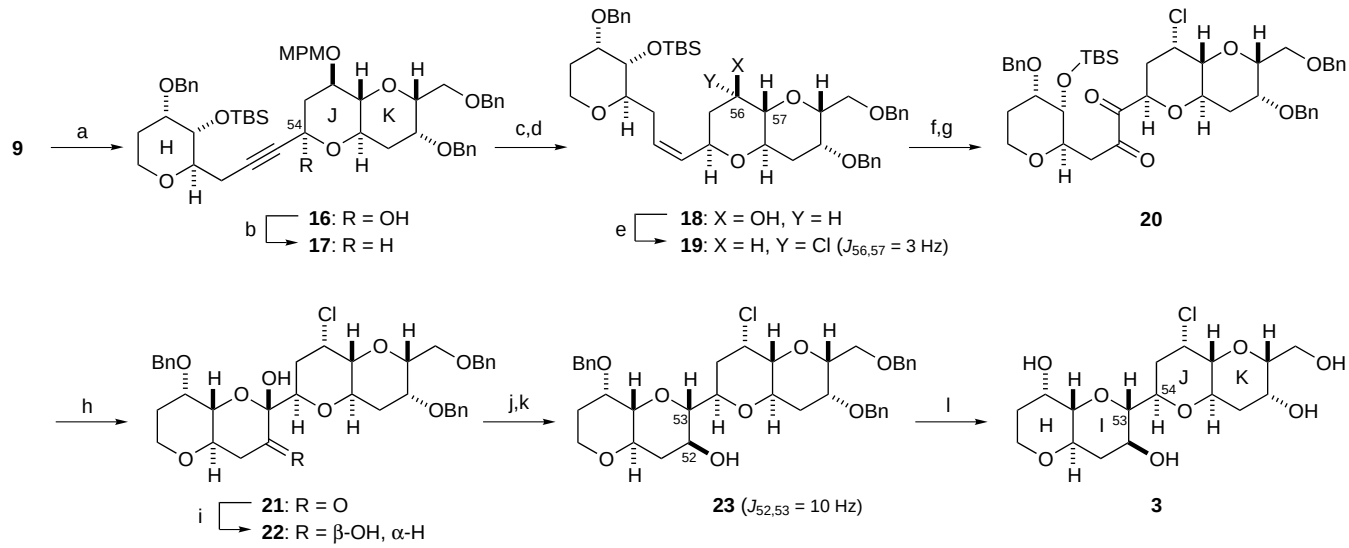
Scheme 1. *Reagents and conditions*: (a) H_2, Pd/C, MeOH, rt; (b) NaOMe, MeOH, rt; (c) $Me_2C(OMe)_2$, PPTS, DMF, rt, quant. (three steps); (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78→0°C; (e) L-selectride, THF, −78°C, 78% (two steps); (f) NaH, BnBr, DMF, 0°C→rt; (g) *p*-TsOH, MeOH, rt, 83% (two steps); (h) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78°C, then TBSOTf; (i) trimethylsilylacetylene, *n*-BuLi, THF–HMPA, −78°C, 77% (three steps); (j) K_2CO_3, MeOH, rt, quant.

(Scheme 3).[12] Lindlar reduction followed by oxidative removal of the MPM group with DDQ produced alcohol **18**. Stereoselective installation of the axial chlorine atom at C56 was achieved by treatment with CCl_4 and Ph_3P.[13] Dihydroxylation of the *cis*-double bond of **19** with OsO_4 and NMO gave a mixture of diols, which was oxidized under Swern conditions to give diketone **20**. Removal of the TBS group with HF·pyr led to the exclusive formation of six-membered hemiketal **21** in 53% yield for the two steps. In this transformation, the corresponding five-membered hemiketal was not produced.[14] DIBALH reduction proceeded stereoselectively to yield alcohol **22**, which was converted to the corresponding methyl acetal and then subjected to $Et_3SiH/BF_3{\cdot}OEt_2$ to yield tetracyclic ether **23**. Finally, removal of the benzyl groups furnished the targeted HI/JK ring model **3** in 65% overall yield for the four steps. The stereochemistry of **3** was unambiguously established by NOE and $^3J_{H,H}$ data.[15]

Examination of the chemical shifts in the ^1H and ^{13}C NMR spectra of **3** thus prepared showed that the observed NMR data matched well with those of *N*-acetylprymnesin-1 (NAPRM1) and *N*-acetylprymnesin-2 (NAPRM2). Especially, the ^1H and ^{13}C NMR signals of the C51-C56 portion correspond extremely well to those of NAPRM1 and 2 (Table 1). Furthermore, the coupling constants, $J_{53,54}=2.5$ Hz, and NOE data of **3** agreed well with those of NAPRM2 (Fig. 1). Thus, the formerly assigned configuration of the HI/JK ring portion was confirmed to be represented by structure **3**. Also, the present results showed that the I/J-ring juncture adopts an exceptional C–C *gauche* conformer, although the precise reason is not yet clear.[16]



Scheme 2. *Reagents and conditions*: (a) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0°C; (b) CSA, MeOH–CH_2Cl_2, rt, 84% (two steps); (c) MMTrCl, Et_3N, DMAP, CH_2Cl_2, rt, quant.; (d) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, rt; (e) L-selectride, THF, −78°C; (f) CSA, MeOH–CH_2Cl_2, rt, 98% (three steps); (g) NaH, BnBr, DMF, 0°C→rt; (h) OsO_4, NMO, acetone–H_2O, rt, 93% (two steps); (i) $NaIO_4$, THF–H_2O, rt; (j) allyltributyltin, $MgBr_2{\cdot}OEt_2$, CH_2Cl_2, −78°C→rt, 90% (two steps) [+14, 7% (two steps)]; (k) *t*-BuOK, MPMCl, *n*-Bu_4NI, THF, 0°C→rt; (l) *n*-Bu_4NF, THF, rt, 91% (two steps); (m) OsO_4, NMO, THF–H_2O, rt; (n) $NaIO_4$, THF–H_2O, rt, 93% (two steps); (o) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, rt, quant.



Scheme 3. *Reagents and conditions*: (a) 9, *n*-BuLi, THF–HMPA, −78°C, then **15**; (b) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −30°C, 68% (two steps); (c) H_2, Lindlar catalyst, MeOH, rt; (d) DDQ, CH_2Cl_2 pH 7.0 phosphate buffer, rt; (e) PPh_3, CCl_4, reflux; (f) OsO_4, NMO, THF–H_2O, rt, 39% (four steps); (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78→0°C; (h) HF·pyr, THF, rt, 53% (two steps); (i) DIBALH, CH_2Cl_2, −78°C; (j) *p*-TsOH, MeOH, 60°C; (k) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C; (l) H_2, $Pd(OH)_2/C$, MeOH, rt, 65% (four steps).

Table 1. Selected ^1H and ^{13}C NMR data of **3**, NAPRM1, and NAPRM2[a]

	3		**NAPRM1**		**NAPRM2**	
No	δ_H (pattern)	δ_C	δ_H (pattern)	δ_C	δ_H (pattern)	δ_C
51	1.57 (ddd, 11.3, 11.3, 11.3)	41.5	1.64	41.3	1.60	41.4
51	2.40 (ddd, 11.3, 4.5, 4.5)		2.45		2.42	
52	3.59 (ddd, 11.3, 9.7, 4.5)	68.0	3.63 (dt, 10, 5)	67.5	3.59	67.8
53	3.48 (dd, 9.7, 2.5)	85.7	3.48 (dd, 10, 2.6)	85.2	3.46	85.6
54	4.47 (ddd, 11.4, 2.5, 2.2)	74.2	4.47	73.8	4.43	74.2
55	2.48 (ddd, 14.7, 11.4, 3.1)	35.7	2.46	35.3	2.47	35.8
55	2.03 (ddd, 14.7, 3.1, 2.2)		2.02		2.03	
56	4.51 (ddd, 3.2, 3.1, 3.1)	60.5	4.46	60.3	4.48	60.3

[a] The spectra were all measured in CD_3OD–C_5D_5N (1:1).

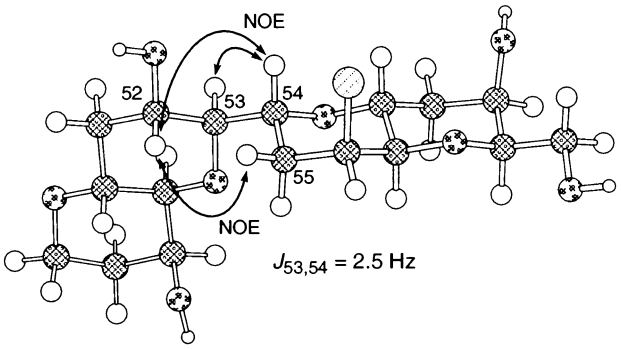


Figure 1.

Acknowledgements

We are grateful for Dr. T. Igarashi, Japan Food Research Laboratories, and Professor M. Satake, Tohoku University, for valuable discussions. This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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15. Selected data for compound **3**: ^{1}H NMR (500 MHz, $CD_3OD/C_5D_5N=1:1$): δ 4.51 (1H, ddd, $J=3.2$, 3.1, 3.1 Hz, 56-H), 4.47 (1H, ddd, $J=11.4$, 2.5, 2.2 Hz, 54-H), 4.29 (1H, ddd, $J=9.4$, 9.3, 4.6 Hz, 58-H), 4.14 (1H, m, 48-H), 4.08 (1H, m, 60-H), 3.95 (1H, dd, $J=11.4$, 6.7 Hz, 62-H), 3.86 (1H, dd, $J=11.4$, 5.0 Hz, 62-H), 3.81 (1H, ddd, $J=11.9$, 11.9, 2.4 Hz, 46-H), 3.53–3.67 (4H, 46-H, 50-H, 52-H, 61-H), 3.48 (1H, dd, $J=9.7$, 2.5 Hz, 53-H), 3.26 (1H, dd, $J=9.3$, 3.2 Hz, 57-H), 2.98 (1H, dd, $J=9.5$, 2.7 Hz, 49-H), 2.48 (1H, ddd, $J=14.7$, 11.4, 3.1 Hz, 55-H), 2.40 (1H, ddd, $J=11.3$, 4.5, 4.5 Hz, 51-H), 2.27 (1H, ddd, $J=12.7$, 4.6, 3.3 Hz, 59-H), 2.03 (1H, ddd, $J=14.7$, 3.1, 2.2 Hz, 55-H), 1.77 (1H, m, 47-H), 1.72 (1H, m, 47-H), 1.67 (1H, m, 59-H), 1.57 (1H, ddd, $J=11.3$, 11.3, 11.3 Hz, 51-H); ^{13}C NMR (125 MHz, $CD_3OD/C_5D_5N=1:1$) δ 85.7 (C53), 83.4 (C61), 82.3 (C49), 80.7 (C57), 74.2 (C54), 71.0 (C50), 69.6 (C58), 68.2 (C60), 68.0 (C52), 66.6 (C48), 64.2 (C46), 63.8 (C62), 60.5 (C56), 41.5 (C51), 39.4 (C59), 35.7 (C55), 35.0 (C47); HRMS (FAB) calcd for $C_{17}H_{27}ClNaO_8$ [$(M+Na)^+$] m/z 417.1292, found 417.1280.
16. In $CDCl_3$ solution, compound **3** preferentially adopted the C–C *anti* conformation around the C53–C54 linkage ($J_{53,54}=8.5$ Hz), presumably due to intramolecular hydrogen bond stabilization between the C52 hydroxyl and the J ring oxygen. Similar conformational properties of the V/W-ring juncture of maitotoxin have been reported, see: Cook, L. R.; Oinuma, H.; Semones, M. A.; Kishi, Y. *J. Am. Chem. Soc.* **1997**, *119*, 7928–7937.