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Synthesis of the JK/LM-ring model of prymnesins, potent hemolytic and ichthyotoxic polycyclic ethers isolated from the red tide alga *Prymnesium parvum*: confirmation of the relative configuration of the K/L-ring juncture

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Abstract—Synthesis of the JK/LM-ring model 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 of the synthetic model with those reported for prymnesins confirmed the reported configurational assignment of the K/L-ring juncture.

Prymnesium parvum is a unicellular alga that blooms in brackish water and causes massive fish kills worldwide. Prymnesin-1 (PRM1, **1**) and prymnesin-2 (PRM2, **2**) were isolated as the causative toxins from the cultured cells of the phytoflagellate. These toxins possess extremely potent hemolytic activity, which is about 5000-fold greater than that of Merck saponin on a molar basis, and also exhibit potent ichthyotoxicity.[3] Their gross structures including partial stereochemistry were determined by Igarashi and co-workers.[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 (ABCDE-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 polycyclic ether domain and four 1,6-dioxadecalin units was determined by extensive NMR analysis. Recently, the absolute configuration of the amino group at C14 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 (Fig. 1).[4] However, the configuration of C76–C85 acyclic portion still remained undetermined.

Based on the coupling constant, $J_{61,62} = 8.5$ Hz, and NOE data, the K/L-ring juncture was inferred to take C60–C63 *anti* conformation. However, a weak NOE between 61-H and 62-H observed in the NOESY spectra of PRM2, along with the intense NOE between 61-H and equatorial oriented 63-Ha in the NOESY spectra of *N*-acetylprymnesin-2 (NAPRM2), suggested the presence of a minor gauche conformer. Hence, stereochemistry of this part should be confirmed by synthetic methods.

We have previously demonstrated that the synthesis of model fragments coupled with NMR *J*-based configuration analysis (JBCA)[5] have successfully elucidated the relative configuration of the acyclic portions of a complex natural product, maitotoxin, the most toxic and largest non-biopolymer.[6] A similar approach was successfully applied to prymnesins, wherein the relative configuration of the I/J- and E/F-ring junctures was confirmed and revised, respectively.[7,8] As part of our studies toward complete stereochemical assignment of prymnesins, we describe herein the synthesis of the JK/LM-ring model **3** for comparison of the NMR data with those of prymnesins, which led to confirmation of the reported stereochemical assignment of the K/L-ring juncture of the natural products.

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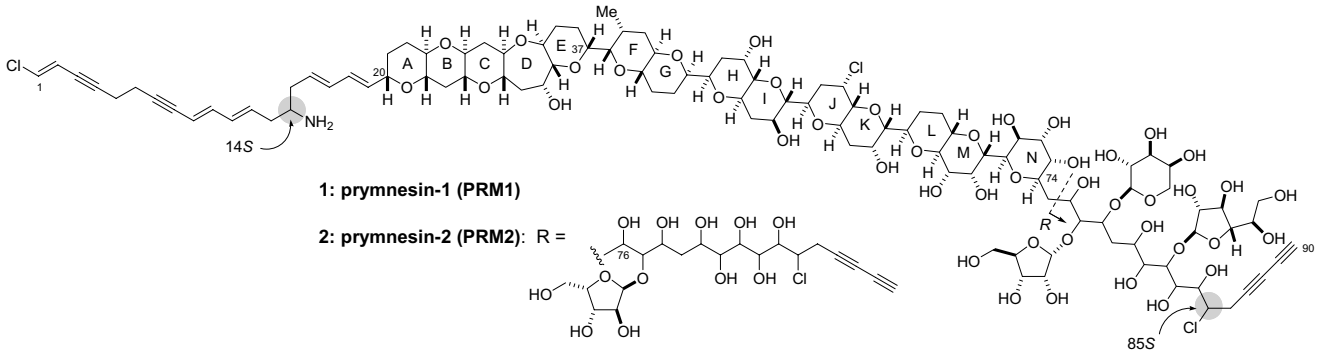
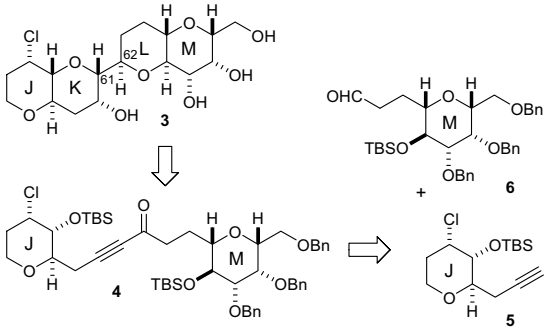


Figure 1. Structures of prymnesin-1 (**1**) and prymnesin-2 (**2**).

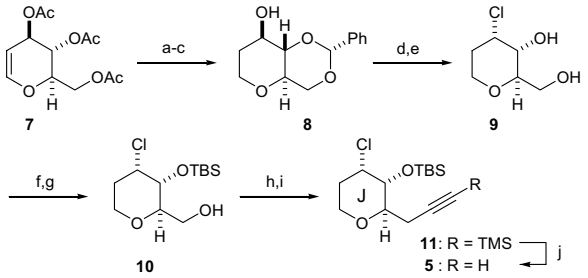
Retrosynthetic analysis of the JK/LM-ring model **3** is outlined in Scheme 1. It was envisioned that target **3** would be derived from alkynyl ketone **4** through reductive cyclization of the K- and L-rings, respectively. The key intermediate **4** would be synthesized via convergent union of the lithium acetylide generated from alkyne **5**, corresponding to the J-ring, and aldehyde **6**, representing the M-ring.

The synthesis of alkyne **5** started with the known alcohol **8**,[9] which was readily obtained from commercially available tri-*O*-acetyl-D-glucal (**7**) in three steps (Scheme 2). Stereoselective installation of the C56[10] chlorine atom was performed by treatment with CCl_4 and PPh_3 in the presence of K_2CO_3, and subsequent removal of the benzylidene acetal provided diol **9** in 89% overall yield. Protection as the TBS ethers, followed by selective removal of the primary TBS group, gave alcohol **10** in 90% yield over two steps. Triflation of the primary hydroxy group and subsequent treatment with lithium trimethylsilylacetylide afforded **11** in 84% yield over two steps. Finally, desilylation with K_2CO_3 provided alkyne **5** in a quantitative yield.

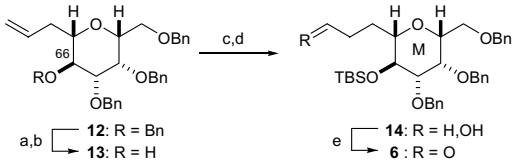
The synthesis of aldehyde **6** commenced with the known compound **12**[11] (Scheme 3). Regioselective removal of the benzyl ether at C66[10] was successfully achieved by the method of Nicotra.[12] Thus, treatment of **12** with iodine to effect debenzylative iodoetherification, followed by reduction with zinc and acetic acid, produced alcohol **13** in 91% yield over two steps. After TBS protection, the terminal olefin was subjected to hydroboration with 9-BBN to give primary alcohol **14** in 92% overall yield.



Scheme 1. Retrosynthesis of model compound **3**.



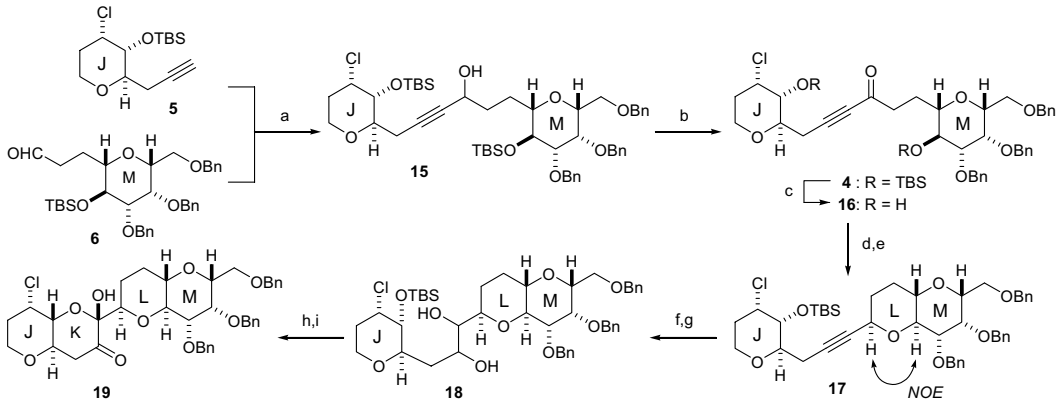
Scheme 2. Reagents and conditions: (a) H_2, Pd/C, EtOAc; (b) NaOMe, MeOH; (c) $PhCH(OMe)_2$, CSA, DMF, 94% (three steps); (d) PPh_3, K_2CO_3, CCl_4, 80 °C, 89%; (e) CSA, MeOH, quant.; (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; (g) CSA, MeOH, 0 °C, 90% (two steps); (h) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C; (i) *n*-BuLi, TMS-acetylene, THF/HMPA, −78 °C, 84% (two steps); (j) K_2CO_3, MeOH, quant.



Scheme 3. Reagents and conditions: (a) I_2, CH_2Cl_2; (b) Zn, HOAc, THF/MeOH, 91% (two steps); (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 98%; (d) 9-BBN, THF; then H_2O_2, $NaHCO_3$, 0 °C, 94%; (e) SO_3·pyridine, DMSO, Et_3N, CH_2Cl_2, 0 °C, 95%.

Oxidation with SO_3·pyridine/DMSO completed the synthesis of the desired aldehyde **6** in 95% yield.

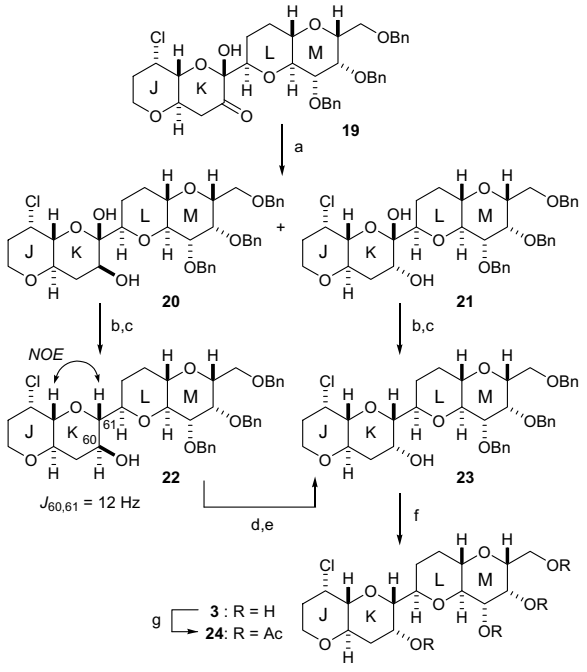
With the requisite fragments in hand, we next carried out their convergent union. The lithium acetylide generated from alkyne **5** (*n*-BuLi, THF/HMPA, −78 °C) was reacted with aldehyde **6** to give propargyl alcohol **15** in 81% yield as a mixture of diastereomers (Scheme 4). Oxidation of **15** under Swern conditions yielded the corresponding ketone **4** in a quantitative yield. Subsequent removal of the TBS ethers was best carried out by the action of HF in acetonitrile to provide dihydroxy ketone **16** in 79% yield. Exposure of **16** to Et_3SiH and BF_3·OEt_2 in acetonitrile effected reductive cyclization to form the L-ring (94%), and the remaining secondary alcohol was protected as the TBS ether to yield **17** in a quantitative yield. The stereochemistry at the newly generated stereo-



Scheme 4. Reagents and conditions: (a) **5**, *n*-BuLi, THF/HMPA, −78 °C; then **6**, 81%; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C → rt, quant.; (c) HF, MeCN, 0 °C, 79%; (d) Et_3SiH, $BF_3{\cdot}OEt_2$, MeCN, 0 °C, 94%; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, quant.; (f) H_2, Lindlar catalyst, quinoline, EtOAc, quant.; (g) OsO_4, NMO, THF/H_2O, 95%; (h) $(CF_3CO)_2O$, DMSO, Et_3N, CH_2Cl_2, −60 °C; (i) HF, MeCN, 0 °C, 91% (two steps).

center was confirmed by NOE, as shown in Scheme 4. Half-reduction of alkyne **17** with Lindlar catalyst, followed by dihydroxylation of the resulting *cis*-olefin, provided diol **18** as an approximately 2:1 mixture of diastereomers (95% yield, two steps). Subsequent oxidation of **18** under the influence of trifluoroacetic anhydride, DMSO, and triethylamine (CH_2Cl_2, −60 °C) led to the corresponding diketone,[13,14] which without purification was subjected to HF in acetonitrile to generate hemiacetal **19** in 91% yield over two steps.

Subsequent treatment of **19** with DIBALH provided β-alcohol **20** and α-alcohol **21** in 65% and 22% yield, respectively (Scheme 5). At this stage, stereochemistry of each compound could not be assigned. Treatment of the major alcohol **20** with *p*-toluenesulfonic acid in methanol at 70 °C produced the corresponding methyl acetal (65% yield), which was then reduced with $Et_3SiH/BF_3{\cdot}OEt_2$ to give tetracyclic ether **22** in 86% yield. The stereostructure of **22** was established by the coupling constant, $J_{60,61} = 12$ Hz, and NOE, as shown. On the other hand, reduction of methyl acetal, derived from **21** (*p*-toluenesulfonic acid, methanol, 70 °C, 75%), with $Et_3SiH/BF_3{\cdot}OEt_2$ afforded the desired **23** in only low yield (22%). Hence, we decided to invert the C60 stereochemistry of **22** by an oxidation/reduction sequence. Dess–Martin oxidation[15] of **22**, followed by reduction with L-Selectride, generated the desired **23** in 83% yield over two steps. Finally, removal of the benzyl ethers under hydrogenolysis furnished the targeted JK/LM-ring model **3** in 92% yield.[16] In addition, the corresponding tetraacetate **24** was prepared under the usual conditions (acetic anhydride, pyridine, room temperature).[17]



Scheme 5. Reagents and conditions: (a) DIBALH, CH_2Cl_2, −78 °C, 65% for **20**; 22% for **21**; (b) *p*-TsOH, MeOH, 70 °C, 65% for **20**; 75% for **21**; (c) Et_3SiH, TMSOTf, CH_2Cl_2, 0 °C, 86% for **22**; 22% for **23**; (d) DMP, CH_2Cl_2, quant.; (e) L-Selectride, THF, −78 °C, 83%; (f) H_2, $Pd(OH)_2/C$, MeOH, 92%; (g) Ac_2O, pyridine, quant.

The JK/LM-ring model **3** and its acetate **24** were subjected to the NMR studies, and their ^1H and ^{13}C NMR chemical shifts for the C59–C64 portion were compared with those of *N*-acetylprymnesin-2 (NAPRM2) and peracetylprymnesin-2 (PAPRM2). The NMR chemical shift values observed for **3** matched the reported values for NAPRM2 (Table 1). In particular, differences in the ^{13}C NMR chemical shifts were within $\Delta\delta = 0.3$ ppm. In addition, the chemical shift values for tetraacetate **24** also corresponded well with those for PAPRM2 (Table 2). Thus, the formerly assigned configuration of the K/L-ring juncture was confirmed to be represented by structure **3**. Also, the coupling constant, $J_{61,62} = 8.4$ Hz, and NOE data of compound **3** (Fig. 2) reproduced those observed for the natural products.[18] These results show that the K/L-ring juncture adopts mostly a C–C *anti* conformation (Fig. 2),[19] although only a minor gauche conformer, which shows NOE between 61-H and 62-H, is present.[18]

In conclusion, we have synthesized the JK/LM-ring model **3** of prymnesins through convergent union of

Table 1. ^{1}H and ^{13}C NMR chemical shifts of C59–C64 regions in *N*-acetylprymnesin-2 (NAPRM2) and model compound **3** (600 MHz, 1:1 C_5D_5N/CD_3OD)[a]

Position	NAPRM2		**3**		$\Delta\delta$[b]	
	^{1}H (pattern)	^{13}C	^{1}H (pattern)	^{13}C	^{1}H	^{13}C
59	1.59	38.7	1.50 (ddd, 12.0, 12.0, 3.0)	38.6	0.09	0.1
	2.23		2.17 (m)		0.06	
60	4.23	66.2	4.19 (brs)	66.3	0.04	−0.1
61	3.20 (dd, 8.5, 2)	84.9	3.25 (brd, 8.4)	84.7	−0.05	0.2
62	3.62 (ddd, 10, 8.5, 2)	77.4	3.64 (m)	77.1	−0.02	0.3
63	1.22	30.3	1.35 (m)	30.3	−0.13	0.0
	1.96		2.04 (brdd, 12.6, 3.0)		−0.08	
64	1.32	30.8	1.41 (m)	30.8	−0.09	0.0
	1.76		1.95 (m)		−0.19	

[a] Referred to internal CHD_2OD (δ 3.31) for ^{1}H NMR and $^{13}CD_3OD$ (δ 50.0) for ^{13}C NMR.
[b] $\Delta\delta = \delta$(NAPRM2) − δ(model **3**).

Table 2. ^{1}H and ^{13}C NMR chemical shifts of C59–C64 regions in peracetylprymnesin-2 (PAPRM2) and compound **24** (600 MHz, $CDCl_3$)[a]

Position	PAPRM2		**24**		$\Delta\delta$[b]	
	^{1}H	^{13}C	^{1}H (pattern)	^{13}C	^{1}H	^{13}C
59	1.61	33.3	1.49 (m)	33.4	0.12	−0.1
	2.21		2.19 (ddd, 13.8, 4.8, 3.6)		0.02	
60	5.08	67.3	5.03 (brs)	67.4	0.05	−0.1
61	3.30	80.4	3.26 (dd, 9.0, 1.2)	80.4	0.04	0.0
62	3.43	74.8	3.38 (brdd, 9.0, 9.0)	74.7	0.05	0.1
63	1.32	28.2	1.28 (m)	28.2	0.04	0.0
	2.12		2.08 (m)		0.04	
64	1.54	28.2	1.53 (m)	28.3	0.01	−0.1
	2.02		2.04 (m)		−0.02	

[a] Referred to internal $CHCl_3$ (δ 7.26) for ^{1}H NMR and $^{13}CDCl_3$ (δ 77.0) for ^{13}C NMR.
[b] $\Delta\delta = \delta$(PAPRM2) − δ(compound **24**).

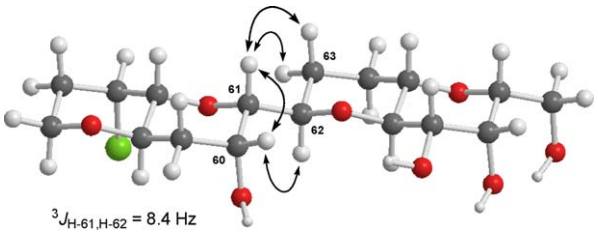


Figure 2. Major conformation for C61–C62 of model compound **3**. Double-headed arrows indicate NOEs.

the J- and M-rings, followed by reductive ring-closure of the K- and L-rings. Comparison of the NMR chemical shifts for model **3** and its tetraacetae **24** with those reported for the natural products and its peracetate derivative allowed the unambiguous confirmation of the assigned relative configuration of the K/L-ring juncture of prymnesins. To establish the complete stereochemistry of the polycyclic portion (C20–C74) of prymnesins, the reported NMR-based configurational assignment of the M/N-ring juncture has to be confirmed by a synthetic approach. Stereochemical assignment of the acyclic polyol portion (C76–C85), together with the absolute configuration and total synthesis of prymnesins, will pose new challenges to synthetic chemists. Further studies toward the complete stereostructure of prymnesins are underway and will be reported in due course.

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16. Data for compound **3**: $[\alpha]_D^{19}$ +54.6 (*c* 0.43, MeOH); IR (film) 3379, 2926, 2871, 1725, 1584, 1304, 1098, 976, 753 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N/CD_3OD) δ 4.47 (d, $J = 2.4$ Hz, 1H), 4.19 (brs, 1H), 4.11 (d, $J = 3.0$ Hz, 1H), 4.06 (ddd, $J = 12.0$, 9.0, 4.2 Hz, 1H), 3.96 (dd, $J = 11.4$, 6.6 Hz, 1H), 3.89 (dd, $J = 11.4$, 5.1 Hz, 1H), 3.77 (brdd, $J = 12.0$, 12.0 Hz, 1H), 3.73 (dd, $J = 9.6$, 3.6 Hz, H), 3.58–3.65 (m, 3H), 3.53 (dd, $J = 9.6$, 9.6 Hz, 1H), 3.25 (dd, $J = 8.4$, 1.2 Hz, 1H), 3.23 (dd, $J = 9.0$, 3.0 Hz, 1H), 3.05 (ddd, $J = 10.5$, 9.6, 4.2 Hz, 1H), 2.17 (m, 1H), 2.13 (m, 1H), 2.04 (brdd, $J = 12.6$, 3.0 Hz, 1H), 1.95 (m, 1H) 1.78 (brd, $J = 15.0$ Hz, 1H), 1.50 (ddd, $J = 12.0$, 12.0, 3.0 Hz, 1H), 1.41 (m, 1H), 1.35 (m, 1H); ^{13}C NMR (125 MHz, C_5D_5N/CD_3OD) δ 84.7, 82.0, 81.2, 81.0, 78.2, 77.1, 74.9, 72.3, 69.3, 66.3, 63.9, 63.5, 60.1, 38.6, 36.1, 30.8, 30.3; HRMS (ESI) calcd for $C_{17}H_{28}O_8Cl$ $[(M+H)^+]$ 395.1473, found 395.1485.
17. Data for compound **24**: $[\alpha]_D^{20}$ +29.4 (*c* 0.40, $CHCl_3$); IR (film) 2916, 2874, 1745, 1431, 1371, 1240, 1129, 1102, 1051, 1029, 980, 755 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.28 (brd, $J = 3.6$ Hz, 1H), 5.03 (brs, 1H), 4.84 (dd, $J = 10.2$, 3.6 Hz, 1H), 4.37 (brd, $J = 3.0$ Hz, 1H), 3.96 (d, $J = 6.6$ Hz, 2H), 3.81 (dd, $J = 12.0$, 10.8 Hz, 1H), 3.77 (dd, $J = 6.6$, 6.6 Hz, 1H), 3.72 (ddd, $J = 12.0$, 9.0, 4.8 Hz, 1H), 3.65 (dd, $J = 12.0$, 4.8 Hz, 1H), 3.38 (brdd, $J = 9.0$, 9.0 Hz, 1H), 3.26 (dd, $J = 9.0$, 1.2 Hz, 1H), 3.22 (dd, $J = 10.8$, 10.2 Hz, 1H), 3.18 (dd, $J = 9.0$, 3.0 Hz, 1H), 3.08 (ddd, $J = 10.8$, 9.0, 4.2 Hz, 1H), 2.19 (ddd, $J = 13.8$, 4.8, 3.6 Hz, 1H), 2.13 (m, 1H), 2.08 (m, 1H), 2.04 (m, 1H), 2.02 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.87 (brd, $J = 15$ Hz, 1H), 1.53 (m, 1H), 1.49 (m, 1H), 1.28 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.5, 170.1, 169.8, 80.4, 78.5, 76.3 (×2), 74.70, 74.66, 71.1, 68.3, 67.4, 66.9, 62.0, 61.8, 57.2, 34.0, 33.4, 28.3, 28.2, 20.9, 20.8, 20.6, 20.5; HRMS (ESI) calcd for $C_{25}H_{35}O_{12}ClNa$ $[(M+Na)^+]$ 585.1715, found 585.1707.
18. A weak NOE observed between 61-H and 62-H in the NOESY spectra of NAPRM2[2] was also reproduced in the model compound **3**.
19. $^2J_{C61,H-62}$ value, −5.1 Hz, also indicated the predominant gauche orientation for C61-O/H-62.