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Stereochemistry of Pteriatoxins A, B, and C

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In 2001, Uemura and co-workers isolated pteriatoxins A, B, and C (PtTXs A, B, and C) from *Pteria penguin*. Recognizing that their ^{1}H NMR characteristics resemble those of PnTX A, they suggested the gross structure for PtTXs A−C (Figure 1), including the stereochemistry, except for the C34 and C2′ positions. It is noteworthy that, although isolated in very minute amounts, PtTXs A−C were reported to exhibit extremely potent and acute toxicity against mice.[1]

In the previous paper, we reported a unified total synthesis of the PtTX class of marine natural products.[2] This synthesis was designed to secure access to all the stereoisomers possible for each member in this class of marine natural products (Scheme 1). In this communication, we report our efforts to establish the stereochemistry of PtTXs A−C, where the availability of all the possible stereoisomers has played an indispensable role.

Stereochemistry of PtTX B and C. All the four C34 and C2′ stereoisomers possible for the PtTX B/C series were synthesized (Scheme 1).[2] On the basis of the ^{1}H NMR and HPLC analyses, each stereoisomer was stereochemically homogeneous. Because natural PtTX B/C was isolated as a 1:1 mixture, comparison of NMR spectroscopic data between the synthetic and natural samples was challenging. Nonetheless, on analysis of the ^{1}H NMR spectrum of the four synthetic stereoisomers, it became evident that several signals, including C32−H, C29−H, C30−H, C28−H, C35−H, and C36−H, are diagnostic to differentiate the C34-*R* series from the C34-*S* series. Among them, the C29−H chemical shift is the most clear-cut marker; in the C34-*R* series, $\delta = 4.663$ ((34*R*,2′*R*)-stereoisomer) and 4.685 ppm ((34*R*,2′*S*)-stereoisomer), whereas in the C34-*S* series, $\delta = 4.527$ ((34*S*,2′*R*)-stereoisomer) and 4.524 ppm ((34*S*,2′*S*)-stereoisomer). With use of these diagnostic signals, we then analyzed the C29−H chemical shift reported for natural PtTXs B ($\delta = 4.61$ ppm) and C ($\delta = 4.54$ ppm), thereby establishing that PtTX B belongs to the C34-*R* series, whereas PtTX C belongs to the C34-*S* series.[3]

With the C34 stereochemistry secured for both PtTX B and PtTX C, we turned our attention to the question of the C2′ configuration. Upon analysis of the ^{1}H NMR spectra shown in Figure 2, the characteristics observed for the C1′ protons appeared to be useful to assign the C2′ stereochemistry. However, this approach met with technical difficulties. We noticed that the NMR profile in question depends on the sample preparation, such as the ionic state and the concentration.[4] Thus, it was critically important to collect the NMR data of the natural and synthetic PtTXs B and C under the identical conditions. Unfortunately, the very limited amount of retained natural PtTXs B/C did not allow us to pursue this approach.

Under this circumstance, we shifted our focus to finding HPLC condition(s) to separate the four stereoisomers of PtTX B/C. In this connection, we should note that natural PtTXs B/C were isolated as an "HPLC-inseparable" mixture.[1] However, our synthetic efforts provided us with all four stereoisomers, which would perhaps make this course of action more feasible. After significant effort, an HPLC method was found, which separated the four stereoisomers (Figure 3).[5] This method allowed us to perform the analysis with much less than 1 μg of samples. With these preparations, we then analyzed the retained natural sample of PtTXs B/C (Figure 3), thereby demonstrating that natural PtTXs B and C match the synthetic (34*R*,2′*R*) and (34*S*,2′*R*) stereoisomers, respectively. It is noteworthy that the LC MS/MS analysis indicates that the natural sample contains a small amount of the (34*R*,2′*S*) and (34*S*,2′*S*) stereoisomers.

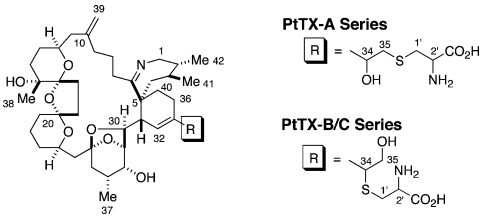
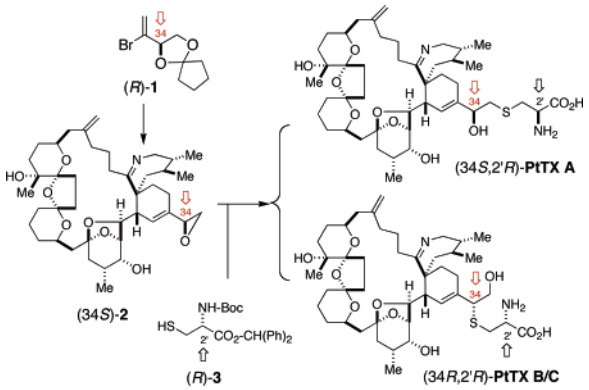


Figure 1. Proposed structure of PtTXs A−C.

Scheme 1. Synthesis of All the C34 and C2′ Stereoisomers Possible for PtTXs A−C (for synthetic details, see our previous paper[2])



		PtTX A	PtTX B/C
β-epoxide series	(*R*)-**1** + (*R*)-**3** →	C34-*S* / C2′-*R*	+ C34-*R* / C2′-*R*
	(*R*)-**1** + (*S*)-**3** →	C34-*S* / C2′-*S*	+ C34-*R* / C2′-*S*
α-epoxide series	(*S*)-**1** + (*R*)-**3** →	C34-*R* / C2′-*R*	+ C34-*S* / C2′-*R*
	(*S*)-**1** + (*S*)-**3** →	C34-*R* / C2′-*S*	+ C34-*S* / C2′-*S*

Stereochemistry of PtTX A. Like the PtTX B/C series, all the possible C34 and C2′ stereoisomers were synthesized (Scheme 1),[2] and their ^{1}H NMR spectra were recorded on a 600 MHz spectrometer in CD_3OD (Figure 4). At the first glance, their ^{1}H NMR spectra might appear too similar to use the ^{1}H NMR spectrum as a diagnostic tool. However, a closer analysis revealed that several NMR characteristics could be useful to differentiate all the

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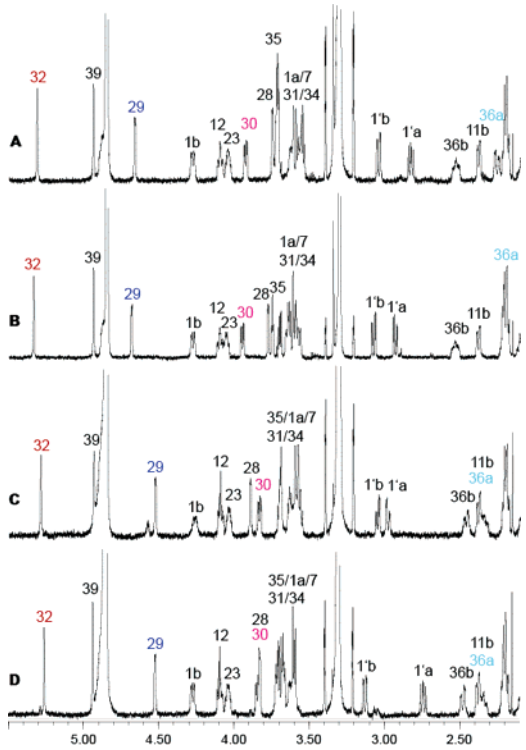

Figure 2. Partial ^{1}H NMR spectrum (750 MHz, CD_3OD) of the four synthetic stereoisomers of the PtTXs B/C series. Panel A: (34*R*,2′*R*)-PtTX B/C. Panel B: (34*R*,2′*S*)-PtTX B/C. Panel C: (34*S*,2′*R*)-PtTX B/C. Panel D: (34*S*,2′*S*)-PtTX B/C.

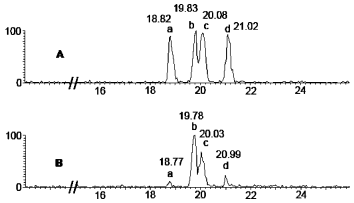

Figure 3. LC MS/MS analysis of PtTXs B/C and their stereoisomers. Panel A: a ca. 1:1:1:1 mixture of the four synthetic PtTX B/C stereoisomers: a = (34*S*,2′*S*)-PtTX C; b = (34*R*,2′*R*)-PtTX B; c = (34*S*,2′*R*)-PtTX C; d = (34*R*,2′*S*)-PtTX B. Panel B: natural PtTX B/C sample.

stereoisomers and consequently to assign the stereochemistry of natural PtTX A. In particular, the ^{1}H NMR characteristics (chemical shifts and resonance patterns) of C34−H, C35−H, C11−H, and C36−H would prove invaluable; see the three areas marked in Figure 4. It should be noted that, like the PtTX B/C series, the proton signals nearby the ionic centers were found to shift significantly depending on the sample preparation and concentration. However, the above-mentioned four characteristics were found to be inert, or at least to be not affected significantly. Focusing on these characteristics, we then compared the reported ^{1}H NMR data[1] with those of the four stereoisomers, thereby demonstrating that the natural PtTX A is the (34*S*,2′*R*) stereoisomer. We then confirmed that the synthetic (34*S*,2′*R*) stereoisomer exhibits a virtually identical overall pattern compared to the ^{1}H NMR spectrum of the natural material deposited in the dissertation of Dr. Noboru Takada.[6]

In summary, we have presented the experimental evidence to establish unambiguously the structure of PtTX A as the (34*S*,2′*R*) stereoisomer and PtTXs B and C as the (34*R*,2′*R*) and (34*S*,2′*R*) stereoisomers, respectively. The availability of all the possible stereoisomers via synthesis has played an indispensable role to the current work. Intriguingly, two C34 stereoisomers (PtTXs B and C) were isolated from the natural source in the PtTX B/C series, whereas only one stereoisomer (PtTX A) was in the PtTX A series.

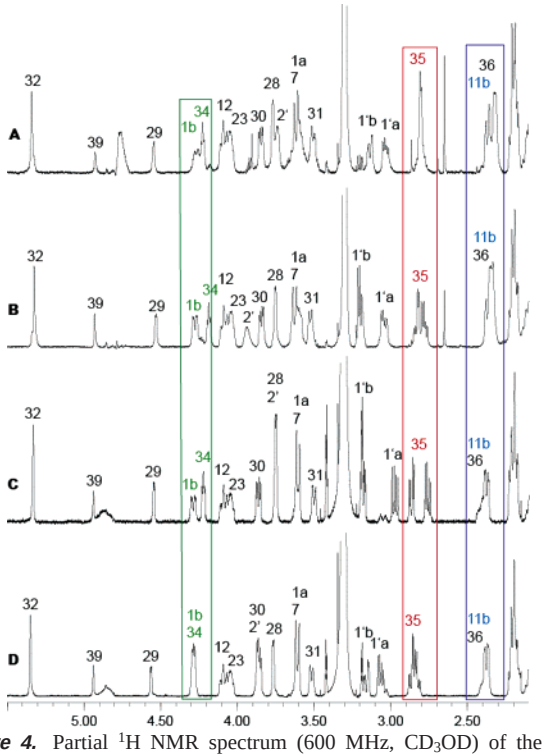

Figure 4. Partial ^{1}H NMR spectrum (600 MHz, CD_3OD) of the four synthetic stereoisomers of the PtTX A series. Panel A: (34*S*,2′*R*)-PtTX A. Panel B: (34*S*,2′*S*)-PtTX A. Panel C: (34*R*,2′*R*)-PtTX A. Panel D: (34*R*,2′*S*)-PtTX A.

With all the possible stereoisomers in hand, we were able to conclude that natural PtTX A is stereochemically homogeneous.[7] Then, the intriguing biosynthetic question is how C34 stereochemistry scrambling occurs in the PtTX B/C series, but not in the PtTX A series. We would suggest (1) that the conjugation of L-cysteine to the macrocyclic core takes place via a C34/C35 epoxide or its biosynthetic equivalent, such as a C34/C35-diol, monophosphate, or monosulfate, and (2) that the conjugation at C35, leading to PtTX A, occurs in a S_N2 fashion, whereas the conjugation at C34 occurs via a S_N1 mechanism due to the resonance stabilization of the involved carbocation.

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Supporting Information Available: ^{1}H NMR spectra and HPLC chromatograms (print/PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Takada, N.; Umemura, N.; Suenaga, K.; Uemura, D. *Tetrahedron Lett.* **2001**, *42*, 3495.

(2) Matsuura, F.; Peters, R.; Anada, M.; Harried, S. S.; Hao, J.; Kishi, Y. *J. Am. Chem. Soc.* **2006**, *128*, 7463.

(3) For details of the ^{1}H NMR assignment, see ref 1.

(4) The concentration effects on the ^{1}H NMR spectra were studied on synthetic (34*S*,2′*R*)-PtTX C; see Supporting Information.

(5) Two additional LC conditions were identified to differentiate partially these stereoisomers. For details, see Supporting Information.

(6) Takada, N. Ph.D. Dissertation, Nagoya University, 2002. Photocopies of the ^{1}H NMR spectra of natural PtTX A and PtTX B/C are included in Supporting Information.

(7) Preliminary mice-toxicity assays were conducted on all the stereoisomers in both PtTX A and B/C series. Interestingly, all of them exhibited acute lethality at the dose of 1 mg/kg. To shed light on the stereochemistry/bioactivity relationship further, we are interested in studying their potency as a Ca^{2+} channel activator.

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