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Unified Total Synthesis of Pteriatoxins and Their Diastereomers

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Outbreaks of human poisoning due to the ingestion of *Pinna* shellfish (*P. muricata* and *P. pectinata*) were recorded in China and Japan, with typical neurotoxin symptoms, such as paralysis, diarrhea, and convulsion. In 1995, Uemura and co-workers isolated pinnatoxin A (PnTX A), one of the major toxic principles responsible for outbreaks of *Pinna* shellfish intoxication, from the shellfish *P. muricata*. They elucidated its gross structure and relative stereochemistry and suggested a biosynthetic pathway.[1] Its unique molecular architecture, accompanied by its pronounced biological activity as a Ca^{2+} channel activator, makes PnTX A an intriguing synthetic target.[2] We previously reported the total synthesis of PnTX A, not only confirming its gross structure and relative stereochemistry, but establishing its absolute configuration (Figure 1).[3]

In 2001, Uemura and co-workers reported two new developments in this area: (1) isolation of PnTXs B and C from the Okinawan bivalve *P. muricata* and (2) isolation of pteriatoxins A, B, and C (PtTXs A, B, and C) from *Pteria penguin*.[4] These new members were isolated in very minute amounts[5] but were reported to exhibit extremely potent and acute toxicity against mice.[6] Considering the similarity in the ^{1}H NMR characteristics of the new toxins to those of PnTX A, Uemura and co-workers suggested the gross structures shown in Figure 1. They proposed that these new alkaloids and PnTX A share the same stereochemistry at the macrocyclic core. However, the C34 stereochemistry of PnTXs B/C and the C34 and C2′ stereochemistry of PtTXs A−C were unassigned.[4]

Our research interests in this area are two-fold: (1) to establish the complete stereochemistry of PtTXs A−C and PnTXs B/C, and (2) to secure an access to stereochemically homogeneous PtTXs A−C[7] and PnTXs B/C, thus permitting unambiguous determination of their individual biological profiles. The naturally occurring PtTXs B/C, as well as PnTXs B/C, were isolated as a mixture and shown to be chromatographically inseparable.[4] Therefore, the individual biological profile of each toxin was not characterized. To achieve our goals, we relied on organic synthesis to access each possible diastereomer in a stereochemically well-defined manner. To synthesize all diastereomers of PtTXs A−C and PnTXs B/C efficiently, we envisioned a unified synthetic plan outlined in Figure 2. This strategy will allow us to synthesize the C34 as well as the C2′ stereoisomers independently and, consequently, secure the complete stereochemistry for all the members of the PnTX/PtTX family. In this paper, we report a total synthesis of all members of the PtTX class of natural compounds.[8]

PtTXs are envisioned to be assembled from three building blocks: dithiane **1**, vinyl iodide **2**, and alkyl iodide **3** (Figure 3).[9] It is worth noting that **1** and **2** are the building blocks used for our PnTX A synthesis, but it is necessary to develop a synthetic route to the building block **3**. The synthesis of the C33−C35 segments **7a** (C34-β series) and **7b** (C34-α series) is summarized in Scheme 1. Hydrolysis of the racemic vinyl bromide diacetate **4**[10] with Amano lipase PS800 furnished a mixture of optically active **5** (optical purity >96%) and **6** (optical purity >96%). On the basis

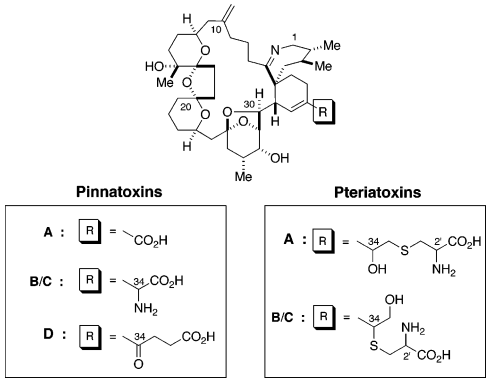


Figure 1. Structure of pinnatoxins and pteriatoxins.

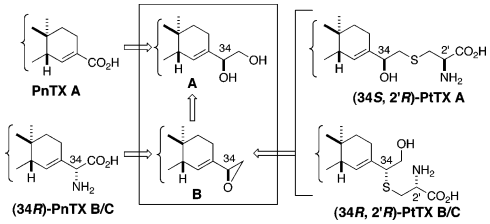


Figure 2. Unified synthesis of the PnTX/PtTX class of marine natural products.

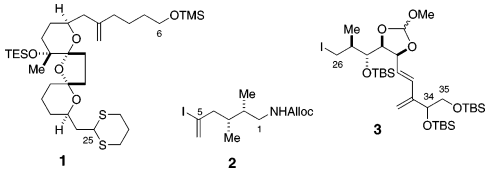
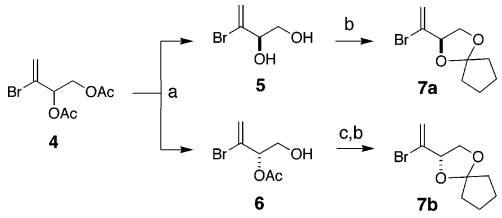


Figure 3. Three building blocks of PtTXs A−C.

Scheme 1 [a]

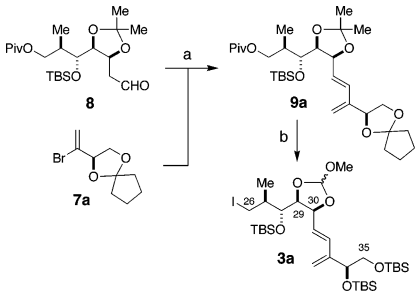


[a] Reagents: (a) Amano lipase PS800 (**5**, 46%; **6**, 41%); (b) cyclopentanone, *p*-TsOH (79%); (c) LiOH (97%).

of literature precedent,[11] their absolute configuration was assigned as indicated, which was further confirmed via chemical correlation with D-(+)- and L-(−)-solketals.[12] This route secured access to antipodes **7a** and **7b** in multigram quantities.

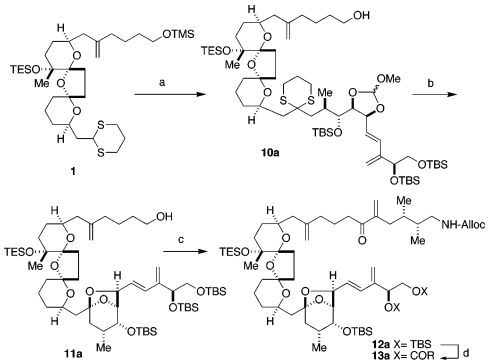
The optically active vinyl bromide **7a** was then elaborated to the C26−C35 segment **3a** (Scheme 2). In this synthesis, the C32−C33 bond was formed via Ni/Cr-mediated coupling[3,13,14] of **7a** and

Scheme 2 [a]



[a] Reagents: (a) (1) **7a**, $NiCl_2$, 86%; (2) Ac_2O, Py, 93%; (3) $Pd(OAc)_2$, $CaCO_3$, 97%; (b) (1) TFA, H_2O, CH_2Cl_2, 91%; (2) Ac_2O, Py, 99%; (3) TFA, H_2O, CH_2Cl_2, 93%; (4) $CH(OMe)_3$, PPTS; (5) K_2CO_3, MeOH; (6) TBSCl, DMAP, 98% over 3 steps; (7) DIBAL, 82%; (8) MeI, DEAD, PPh_3, 88%.

Scheme 3 [a]



[a] Reagents: (a) (1) **3a**, *t*-BuLi, HMPA; (2) K_2CO_3, MeOH, 73% over 2 steps; (b) (1) PPTS; (2) K_2CO_3, MeOH, 73% over 2 steps; (3) PIFA, 74%; (c) (1) SO_3·Py, DMSO, 83%; (2) **2**, $NiCl_2$, $CrCl_2$, 84%; (3) Dess−Martin oxidation, 79%; (d) (1) HF·Py, Py; (2) RCOCl, NEt_3.

8,[3] to furnish a diastereomeric mixture of allylic alcohols, which, upon acylation and Pd-mediated elimination, afforded diene **9a**. At this stage, it was necessary to convert the C29/C30 acetonide to the more labile ortho ester protecting group (**3a**) due to our inability to deprotect the acetonide at later stages in the synthesis.

Coupling of iodide **3a** with dithiane **1** then furnished the C6−C35 portion of the PtTXs in high yield (Scheme 3). Elaboration to the requisite Diels−Alder precursor **12a** commenced with removal of the ortho ester and dithiane deprotection with concomitant formation of the C25−C30 bicycloketal. Oxidation of the C6 hydroxyl then permitted installation of the C1−C5 moiety via a Ni/Cr-mediated coupling.[3,13] Alternatively, introduction of the C1−C5 moiety, followed by the C25−C30 bicycloketal formation, also afforded **12a**, but the material throughput via the sequence of reactions shown in Scheme 3 proved superior.[15]

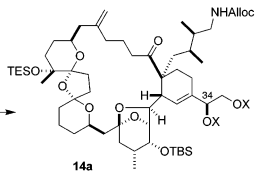
The next stage of synthesis was the crucial intramolecular Diels−Alder reaction to form the carbo-macrocycle. Previously, we successfully relied on an intramolecular Diels−Alder reaction to construct the macrocycle of PnTX A.[3] However, it is worth noting that the diene used in that series was conjugated with a *tert*-butyl ester, which exhibited a high tendency for self [4 + 2] cycloaddition, and therefore it was necessary to handle the diene only as a dilute solution. To the contrary, we anticipated, and indeed found, that the diene in the current series shows no tendency of self [4 + 2] cycloaddition.

We first studied the intramolecular Diels−Alder reaction on substrate **12a** in the C34-β series. Under thermal conditions (dodecane, 170 °C), **12a** slowly cyclized to give a 0.8:1.0:0.8 mixture of the intramolecular Diels−Alder products in 78% combined yield (Table 1). The two *exo*-products were chemically

Table 1. Intramolecular Diels−Alder Reactions[a]

C34 β-Series

12a : X=TBS
13a-i : X=Bz
13a-ii : X=2-Naphthoyl
13a-iii : X=(*p*-MeO)Bz
13a-iv : X=(*o*-MeO)Bz
13a-v : X=Ac
13a-vi : X=(*p*-MeO_2C)Bz
13a-vii : X=(*R*)-MTPA
13a-viii : X=(*S*)-MTPA



14a

	Temp	A	B	C	D	E
12a	170 °C	0.8 : 1.0 : 0.8	78%	24%	2.3 : 1.0	0.8 : 1.0
13a-i	160 °C	1.4 : 1.0 : 0.3	>90%	NA	8.0 : 1.0	1.4 : 1.0
13a-ii	160 °C	1.6 : 1.0 : 0.4	73%	37%	6.5 : 1.0	1.6 : 1.0
13a-iii	160 °C	5.2 : 1.0 : 1.2 : 2.0	88%	51%	1.9 : 1.0	5.2 : 1.0
13a-iv	160 °C	3.6 : 1.0 : 1.5	50%	NA	3.1 : 1.0	3.6 : 1.0
13a-v	160 °C	1.9 : 1.0 : 1.1 : 0.3	93%	NA	2.1 : 1.0	1.9 : 1.0
13a-vi	160 °C	1.3 : 1.0 : 0.6	97%	41%	3.8 : 1.0	1.3 : 1.0
13a-vii	160 °C	1.0 : 1.0 : 1.0	>90%	NA	2.0 : 1.0	1.0 : 1.0
13a-viii	160 °C	1.0 : 1.0 : 1.0	>90%	NA	2.0 : 1.0	1.0 : 1.0

C34 α-Series

12b: X=TBS; **13b-i** : X=Bz;
13b-ii : X=(*p*-MeO)Bz;
13b-iii : X=Ac; **13b-iv** : CHO

14b

	Temp	A	B	C	D	E
12b	185 °C	1.1 : 1.0 : 0.8	52%	20%	2.6 : 1.0	1.1 : 1.0
13b-i	160 °C	3.6 : 1.0 : 1.0	48%	30%	4.6 : 1.0	3.6 : 1.0
13b-ii	160 °C	1.0 : 1.1 : 3.1	>90%	20%	0.7 : 1.0	0.9 : 1.0
13b-iii	160 °C	2.4 : 1.3 : 1.0	80%	36%	3.7 : 1.0	1.8 : 1.0
"	130 °C	3.5 : 2.1 : 1.0	NA	NA	5.6 : 1.0	1.7 : 1.0
13b-iv	160 °C	1.4 : 1.5 : 1.0	76%	NA	2.9 : 1.0	0.9 : 1.0

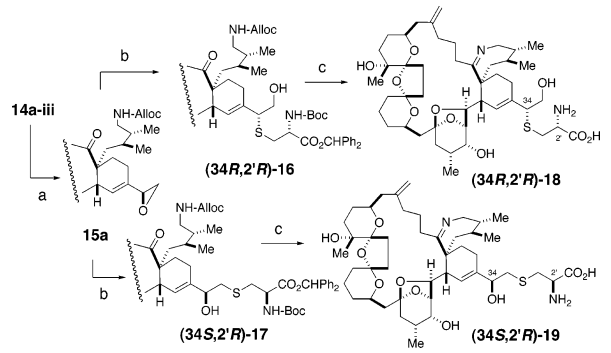
[a] **A**: product ratio (*exo*-desired:*exo*-undesired:*endo*-1:*endo*-2). **B**: combined yield. **C**: isolated yield of desired *exo*-product. **D**: *exo*/*endo* ratio. **E**: *exo*-facial selectivity (desired:undesired).

correlated with the two *exo*-products in the previous synthesis,[3,12] thereby establishing their stereochemistry. However, the stereochemistry of *endo*-product(s) remains to be established. Upon changing the C34/C35 protecting groups from TBS to acyl groups, the cycloaddition took place noticeably faster.[16,17] Interestingly, the *exo*/*endo*-selectivity was found to depend sharply on the acyl protecting group, with the benzoate giving the best ratio. The facial selectivity of *exo*-addition was found also to depend on the protecting group, with the *p*-methoxybenzoate giving the best ratio. On the balance of the two selectivities, we chose the *p*-methoxybenzoate substrate for preparative purposes and obtained the desired product **14a-iii** in 51% isolated yield.

The overall trend of the intramolecular Diels−Alder reaction in the C34-α series was similar to that in the β series, except that the chemical yield in the α series was lower than that in the corresponding β series. In this series, the temperature effect on the *exo*/*endo*-selectivity and the facial selectivity of *exo*-products was tested on the acetate substrate **13b-iii**. Interestingly, on lowering the reaction temperature, the *exo*/*endo*-selectivity was noticeably improved, whereas the facial selectivity of *exo*-products remained virtually unchanged.

We next planned to introduce the cysteine moiety via epoxide **15a**, which was in turn prepared from the C34/C35-diester **14a-iii**. Considering the allylic nature of epoxide, also supported by literature precedents,[18] we anticipated that ring opening could be achieved preferentially at the secondary center in a S_N2 fashion, that is, **15a** → **16**. Experimentally, treatment of **15a** with the anion derived from *N*-Boc-L-Cys(SH)-$OCHPh_2$ yielded a 2:1 mixture of (34*R*,2′*R*)-**16** and (34*S*,2′*R*)-**17** in an approximately 85% combined yield. Critically, both **16** and **17** were found to be free from contamination of the corresponding C34 or C2′ stereoisomers,[19] thereby demonstrating that the epoxide ring opening took place in a S_N2 fashion, and therefore, their stereochemistry was assigned as indicated. Similarly, when we employed the anion derived from *N*-Boc-D-Cys(SH)-$OCHPh_2$, **15a** afforded a 2:1 mixture of two products corresponding to (34*R*,2′*S*)-**16** and (34*S*,2′*S*)-**17** in com-

Scheme 4 [a]



[a] Reagents: (a) (1) K_2CO_3; (2) HF·Py, Py, 74% over 2 steps; (2) TsCl; (3) K_2CO_3, 78% over 2 steps; (b) *N*-Boc-L-Cys(SH)-OCHPh$_2$ (an inseparable mixture of **16** and **17**), 85%; (c) (1) $Pd(PPh_3)_4$, AcOH, 72%; (2) 1,3,5-(*i*-Pr)$_3C_6H_2CO_2H$/Et_3N salt 80 °C, xylene; (3) TFA, CH_2Cl_2, followed by HPLC separation of **18** and **19**.

parable yield. Once again, both products were found to be stereochemically homogeneous.[19]

In the pinnatoxin A synthesis, the Diels−Alder product was converted to the natural product in three steps: (1) $Pd(PPh_3)_4$, AcOH (deprotection of the Alloc group), (2) 200 °C, 1×10^{-2} mmHg (imine cyclization), and (3) TFA/CH_2Cl_2 (deprotection of the *t*-Bu ester).[3] For the present series, the Alloc deprotection smoothly took place under the same conditions, to give the desired amino ketone. Disappointingly, the resultant amino ketone did not survive under the thermolysis conditions. In our earlier work, we found imine formation under traditional, weakly acidic conditions to be unsuccessful. For example, in the PnTX A synthesis, reaction in the presence of AcOH and Et_3N did not promote imine cyclization at room temperature, whereas undesired *N*-acetylation was observed at elevated temperatures. However, the instability of amino ketone in this series under the original thermolysis conditions led us to revisit the imine cyclization under weakly acidic conditions. Specifically, we searched for weakly acidic conditions under which the undesired *N*-acylation might be avoided or suppressed; in particular, we focused on combinations of sterically congested carboxylic acids and tertiary amines and eventually found that the 2,4,6-(*i*-Pr)$_3C_6H_2CO_2H$[20]/Et_3N salt meets our needs.

To complete the total synthesis, the only remaining task was to remove the protecting groups of the cysteine moiety. For two specific reasons, we chose *N*-Boc-Cys-OCHPh$_2$. First, our previous work[3] demonstrated that all the functional groups present in PnTX A, including the seven-membered imine, survive under the TFA/CH_2Cl_2 (deprotection of the *t*-Bu ester) conditions. Second, the combination of these two specific protecting groups ensured that the protecting group of the carboxylic acid is cleaved prior to that of the amine.[21] Upon treatment with TFA in CH_2Cl_2 at room temperature, both of the protecting groups were smoothly removed. Finally, preparative LC allowed separation and isolation of pure synthetic (34*S*,2′*R*)-PtTX A and (34*R*,2′*R*)-PtTX B/C.[22] Overall, the epoxide **15a** furnished two out of the four possible stereoisomers at C34 and C2′ for both the PtTX A and PtTX B/C series.

Applying the same synthetic sequence on the building block **7b**, we were able to synthesize the remaining stereoisomers at C34 and C2′ for both the PtTX A and PtTXs B/C series. However, comparison of NMR spectroscopic data between the synthetic and natural samples did not lead us to the conclusion. In a subsequent paper, we report our efforts to establish the stereochemistry of natural PtTXs A−C, demonstrating that the availability of all possible stereoisomers is essential to rigorously address the problem.[23]

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) A 1:1 mixture (0.3 mg) of PnTXs B/C was isolated from 21 kg of the viscera of *Pinna muricata*. PtTX A (20 μg) and PtTX B/C (8 μg as a 1:1 mixture) were isolated from 82 kg of the viscera of *Pteria penguin*.
(6) Reported LD_{99} in ref 4: PnTXs B/C = 22 μg/kg; PtTX A = 100 μg/kg; PtTXs B/C = 8 μg/kg).
(7) The stereochemical homogeneity of PtTX A is discussed in a subsequent paper, see ref 23.
(8) A total synthesis of PnTXs B and C from the Diels−Alder products **14a-iii** and **14b-iii**, respectively, will be reported elsewhere.
(9) The building blocks dithiane **1** and vinyl iodide **2** were reported in ref 3. We adopted the reported synthesis for preparation of vinyl iodide **2**. However, we have made substantial modifications on the synthesis of dithiane **1**; for details, see Supporting Information.
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(15) The alternative sequence of reactions for transformation from **10a** to **12a** were (a) 1, PIFA; 2, TPAP, NMO; 3, $NiCl_2$, $CrCl_2$, **2**; 4, Dess−Martin oxidation, 27% over 4 steps; (b) 1, PPTS; 2, K_2CO_3, MeOH; 3, PPTS, 63% over 3 steps.
(16) The substrates **12** and **13** did not survive in the presence of Lewis acids or in the absence of Sumilizer.
(17) The intramolecular Diels−Alder reaction of **12a** and **12b** was not observed below the indicated temperature.
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(21) We assumed, and indeed observed, that acid-promoted deprotection of the *t*-Bu ester requires much more forcing conditions, once the amino-protecting group is hydrolyzed.
(22) Column: YMC-Pack ODS-A, 250 × 10 mm. Solvent: H_2O/CH_3CN = 3/1 (v/v) containing 0.1% TFA. Flow rate: 2 mL/min, isocratic. Detection: 216 nm.
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