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# Total synthesis and *in vivo* evaluation of 8-deoxypumiliotoxin 193H

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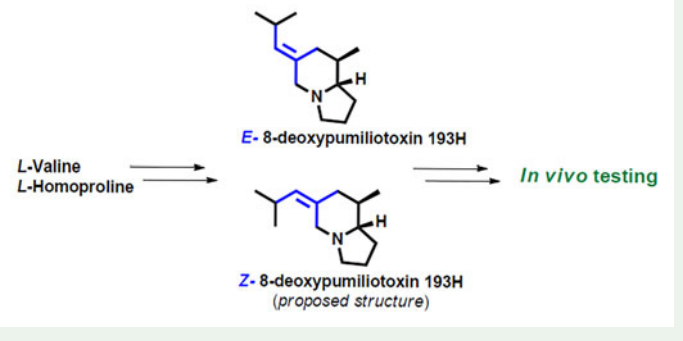
**ABSTRACT**

The total synthesis of both the double bond isomers of indolizine alkaloid 8-deoxypumiliotoxin 193H has been accomplished. Both the double bond isomers Z-4 and E-4 induced convulsions and inhibited neuro-muscular activity at a dose of 25 mg/kg after intraperitoneal injection in mice. The lethal dose of Z-4 and E-4 was 100 mg/kg, indicating that 8-deoxypumiliotoxin 193H is 10-times less toxic than the known pumiliotoxin (+)-251 D.

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Total synthesis; stereoselectivity; in vivo studies; deoxypumiliotoxin 193H

## 1. Introduction

Amphibians possess a broad range of structurally unique naturally occurring compounds (Simmaco et al. 1998; Daly 1998; Daly et al. 2004; Daly et al. 2005; Pukala et al. 2006; Xu and Lai 2015; Rodríguez et al. 2017; Dennison et al. 2018) with intriguing biological properties, including anticancer (Fornari Baldo et al. 2012), antimicrobial (Simmaco et al. 1998; Cunha Filho et al. 2005; Wu et al. 2011; Wang et al. 2013; Dennison et al. 2018), antifungal (Artika et al. 2015) and cardiotonic (Daly et al. 1985) activities. The most frequently used source of these natural products is the skin secretion of frogs that populate the forests of South America, Africa and Australia. These amphibians were historically used to prepare poisoned arrows for hunting and warfare

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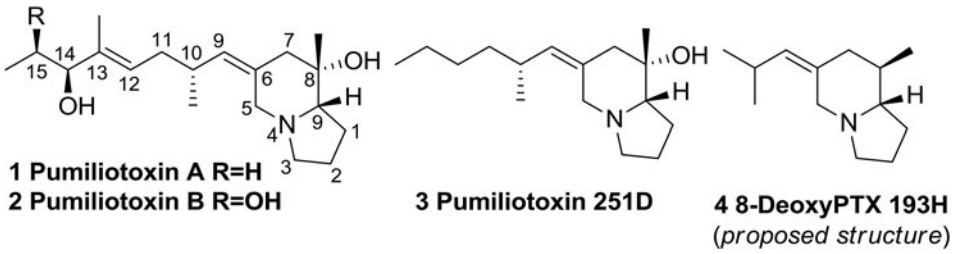
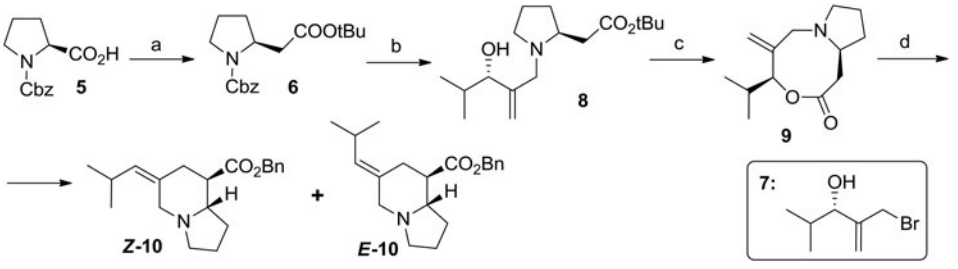



Figure 1. Representative members of the PTX family.

(Jones 2007). Most of these alkaloids are sequestered unchanged from dietary arthropods such as mites, ants, beetles, and millipedes (Saporito et al. 2009). Today, more than 800 alkaloids have been characterized from the skins of dendrobatid frogs (Daly et al. 2005). A broad family of indolizine alkaloids found in dendrobatid frogs are the pumiliotoxins (PTX) (Pelletier 1999; Daly et al. 2005; Michael 2016). The first members of this family, PTX A (**1**) and B (**2**), were isolated by Daly in the 1960s (Daly and Myers 1967); however, their structure remained unclear for almost 2 decades. In the 1980s, the isolation and X-ray characterization of a simpler indolizine alkaloid PTX 251 D (**3**) (Daly et al. 1980) allowed the resolution of the also structures of **1** and **2**. The total syntheses of PTX were pioneered by Overman (Overman and Bell 1981; Overman and Goldstein 1984; Overman and Lin 1985; Franklin and Overman 1996) in the 1980s and are still ongoing (Suryavanshi et al. 2014; Chou et al. 2014; Correia et al. 2016; Qu and Helmchen 2017). PTX is known to inhibit sodium and potassium channels (Gusovsky et al. 1992; Vandendriessche et al. 2008; Daly et al. 1990). The estimated minimum lethal dose of PTX A (**1**) and B (**2**) in mice is 2.5 and 1.5 mg/kg, respectively (Daly and Myers 1967), and for PTX (+)-251 D (**3**) the lethal dose is 10 mg/kg. Moreover, this effect is strongly dependent on the stereochemistry and substitution pattern of the molecule (Daly et al. 1990; Daly et al. 2003). Additionally, PTX (+)-251 D (**3**) induces convulsions, apparent pain at the site of injection and pronounced long-lasting hyperactivity in mice (Daly et al. 2003), while PTX A (**1**) and B (**2**) inhibit nerve-muscle activity (Daly and Myers 1967) (Figure 1).

A common structural motif of PTXs is an octahydroindolizine core that possess an alkylidene substituent at the C-6 position. One of the simplest members of the PTX alkaloid family is 8-deoxyPTX 193H (**4**), which is found in *Scheloribates azumaensis* mites that populate Japan (Takada et al. 2005). This compound, however, has never been isolated, and its structure was proposed only on the basis of a GC-MS fragmentation pattern and analogy to other PTX family members (Takada et al. 2005). Despite its structural simplicity, 8-deoxyPTX 193 H (**4**) contains most of the characteristic structural elements of PTX: an exocyclic *Z*- configuration double bond and an indolizine core with 2 chiral centres, making it an interesting target from a medicinal chemistry perspective. Over the past decades, a number of different approaches have been reported for the assembly of the indolizine core of PTX (Fox et al. 1991; Franklin and Overman 1996; Lin et al. 1996; O'Mahony et al. 2004; Pinho et al. 2013); however, a stereoselective introduction of the alkylidene moiety at the C-6 position poses a considerable challenge. The putative 8-deoxyPTX 193H (**4**) was first synthesized by our group in 2015 (Smits and Zemribo 2015) followed by a recent synthesis by Okada (Okada et al.




Scheme 1. Reagents and conditions: (a) i) NMM, EtOCOCl, THF, then $CH_2N_2$, rt, 75%; ii) $CF_3COOAg$, $Et_3N$, tBuOH, rt, THF, rt, 72%; (b) i) $H_2$, 10% Pd/C, EtOH, rt; ii) **7**, DIPEA, THF, rt, 80% in 2 steps; (c) i) TFA, DCM, rt; ii) HBTU, DMAP, 0.01 M, DCM, rt, 83% in 2 steps; (d) $Bu_2BOTf$, $Me_2NEt$, 1 h at 0 °C, then 1 h at rt, then 1 h at 50 °C (MW), then BnOH, HBTU, 39% for ***Z*-10**, 9% for ***E*-10**.

2018), but the *in vivo* activity has never been evaluated. Herein, we report the total synthesis of both the double bond isomers of 8-deoxyPTX 193H (**4**). The rota-rod, traction and chimney tests were utilized and body temperature was measured to assess toxicity and determine the effects of both isomers on muscle strength and coordination in mice.

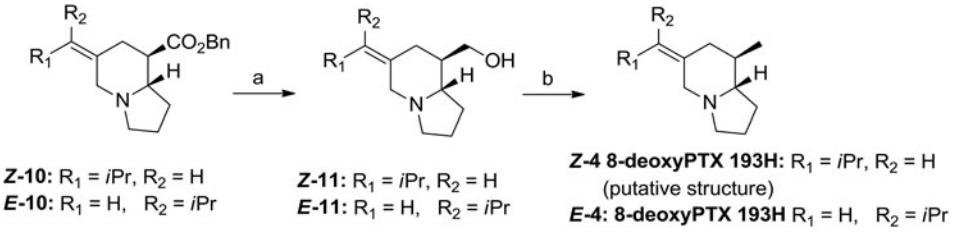
## 2. Results and discussion

Our approach towards the total synthesis of 8-deoxyPTX 193H (**4**) (Smits and Zemribo 2015) was based on a stereoselective Ireland–Claisen rearrangement developed earlier by our group (Smits et al. 2015; Smits and Zemribo 2013) (Scheme 1).

Our synthetic studies towards the synthesis of 8-deoxyPTX 193H (**4**) began with an Arndt-Eistert homologation of the commercial proline **5** giving the desired homoproline derivative **6** in a 54% yield over 2 steps. The Cbz-protecting group in **6** was further cleaved by palladium catalysed hydrogenation, and the intermediate was alkylated with allyl bromide **7** (Honda et al. 1987; Shklyaruck and Matiushenkov 2011) furnishing the homoproline ester **8** in an 80% yield over 2 steps. Next, the ester **8** was converted to the eight-membered lactone **9** first by cleavage of the *tert*-butyl ester, followed by a macrolactonization under high dilution conditions. With the lactone **9** in hand, the stage was set for the crucial step of this total synthesis, a stereoselective Ireland–Claisen rearrangement. Despite our attempts to optimize this step (Smits and Zemribo 2015), the desired indolizines **10** were obtained in moderate yields and ~1:4 *E*-/*Z*- selectivity; however, we were able to separate the double bond isomers ***Z*-10** and ***E*-10** by flash column chromatography and focus on the total synthesis (Scheme 2).

The synthesis of 8-deoxyPTX 193H ***Z*-4** and its double bond isomer ***E*-4** was accomplished in a 2-step sequence. First, the esters ***Z*-10** and ***E*-10** were reduced to the corresponding alcohols ***Z*-11** and ***E*-11**, followed by tosylation and reduction of the intermediate tosylates with a large excess of Superhydride®. Using this methodology >50 mg of both 8-deoxyPTX 193H ***Z*-4** and ***E*-4** were synthesized and further used the *in vivo* studies in mice.

To measure the effects of compounds on muscle strength and coordination, we utilized the rota-rod, traction and chimney tests and measured body temperature in




Scheme 2. Reagents and conditions: (a) DIBAL-H, DCM, -78 °C, 90% for ***Z*-11**, 81% for ***E*-11**; (b) TsCl, cat. DMAP, DIPEA, then Superhydride®, THF, 50% for ***Z*-4**, 54% for ***E*-4**.

Table 1. The toxicological profile of ***Z*-4** and ***E*-4** in mice.

| Compounds | Dose, mg/kg | Convulsive behaviour | Muscle strength and coordination | Body temperature changes | Mortality |
|---|---|---|---|---|---|
| ***Z*-4** | 10 | 0/1 | 0/1 | 0/1 | 0/1 |
| | 25 | **1/1** | **1/1** | 0/1 | 0/1 |
| | 50 | **2/2** | **2/2** | **1/2** | 0/2 |
| | 100 | **1/1** | **1/1** | **1/1** | **1/1** |
| ***E*-4** | 10 | 0/2 | 0/2 | 0/2 | 0/2 |
| | 25 | **2/2** | 1/2 | 0/2 | 0/2 |
| | 50 | **2/2** | **2/2** | **1/2** | 0/2 |
| | 100 | **1/1** | **1/1** | **1/1** | **1/1** |

The compounds were administered i.p. at doses of 10, 25, 50 and 100 mg/kg. The effects were observed 30, 60, 120, 180 and 240 min after administration. The data are expressed as a n animals with effects/total animal n.

mice (see at Supplementary data) (Dambrova et al. 2008). The effects of compounds were evaluated at 30, 60, 120, 180 and 240 min after intraperitoneal (i.p.) administration at doses of 10, 25, 50 and 100 mg/kg.

***Z*-4** and ***E*-4** induced convulsion at a dose of 25 mg/kg. The convulsive behaviour in mice started approximately 5 min after the compound administration and lasted 15 min. The compounds also induced seizures at higher doses of 50 and 100 mg/kg. The inhibitory activity of ***Z*-4** and ***E*-4** on muscle strength and coordination were observed at a dose of 25 mg/kg in the first 30 min and at a dose of 50 mg/kg in the first 60 min period. In addition, both isomers decreased the rectal temperature at a dose of 50 mg/kg during the first 60 min. The administration of ***Z*-4** and ***E*-4** at a dose of 10 mg/kg did not bring about any effects. After i.p. administration of ***Z*-4** and ***E*-4** at a dose of 100 mg/kg the mice died during the first 10 min (Table 1).

## 3. Conclusions

In summary, ***Z*-4** and ***E*-4**, the double bond isomers of the putative 8-deoxyPTX 193H, were synthesized using an Ireland–Claisen rearrangement as the stereochemistry determining step. Both isomers ***Z*-4** and ***E*-4** administered at equal doses induced convulsions, inhibited neuro-muscular responses and reduced body temperature in mice. The lethal dose of 8-deoxyPTX 193H was 10-fold higher compared to PTX (+)-251 D. The less toxic properties of 8-deoxyPTX could be attributed to a simplified alkylidene substituent at the C-6 position compared to PTX 251 D as well as PTX A and B.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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