SYNTHESIS AND CONFIRMATION OF THE STRUCTURES OF POLONICUMTOXINS A, B AND C

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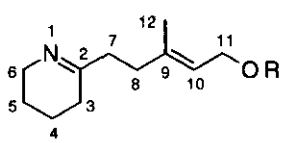
Abstract- Total syntheses of polonicumtoxins A (**1**), B (**2**), and C (**3**) have been completed. The key step of the syntheses involves an aza-Wittig reaction for the construction of the cyclic ketimine. The synthetic compounds were found to be identical in all respects with the natural products, thus confirming their assigned structures.

Polonicumtoxins A (**1**), B (**2**), and C (**3**) are cyclic ketimine toxins isolated from the freshwater dinoflagellate *Peridinium polonicum*, which occasionally blooms in lakes and drinking water reservoirs.[3] These compounds exhibit extremely potent toxicity toward fish, making the dinoflagellate blooms a serious environmental problem. The ichthyotoxicity of **1** (*Oryzias latipes*, LC_{99}, 13 ppb) is comparable with that of brevetoxin B (LC_{99}, 16 ppb),[4] a well-known marine dinoflagellate toxin. Compounds (**1**) and (**3**) are also lethal to mice, albeit at a higher concentration (i.p. LD_{99}, **1**: 1.5 mg/kg; **3**: 2.0 mg/kg; **2** was not tested).[3] The probable structures of **1**, **2**, and **3** were previously elucidated.[3] However, their limited availability coupled with instability prevented unambiguous determination of the olefin geometry by NOE experiments and assignments of the ^{13}C NMR signals (C4 and C5 of **1**, **2**, and **3**, and C2 and C1' of **1**) based on the ^{13}C-^{1}H correlation. Similar tetrahydropyridyl alkaloids such as γ-coniceine and the ant toxin 2-(pentene-1-yl)-3,4,5,6-tetrahydropyridine,[5-7] have been isolated from other natural sources. While the polonicumtoxins have strong bioactivity, the details of their mechanism of action have not been fully studied. We have examined several routes to these natural products and describe here a general approach that has culminated in the synthesis of polonicumtoxins A, B, and C (**1**, **2**, and **3**), providing confirmation of their previously proposed structures.



polonicumtoxin A (**1**): R = $\overset{1'}{C}O\overset{2'}{C}H_2\overset{3'}{C}H{=}\overset{4'}{C}H_2$

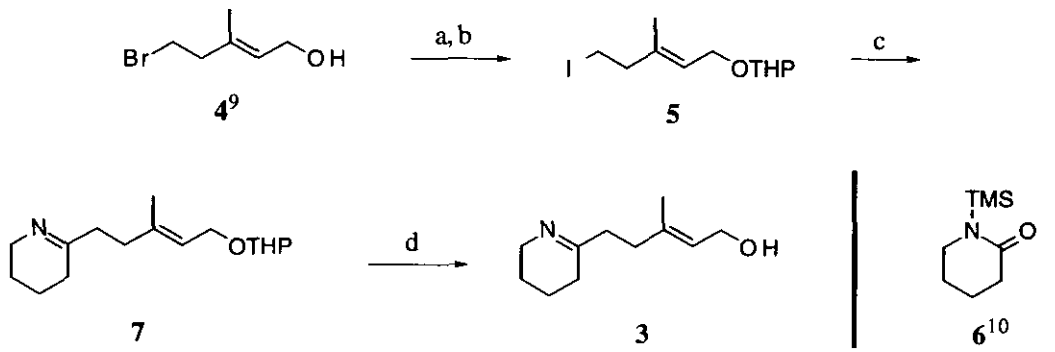
polonicumtoxin B (**2**): R = $\overset{1'}{C}O\overset{2'}{C}H_3$

polonicumtoxin C (**3**): R = H

Synthesis of Polonicumtoxin C (3) (Route 1)

The objective of the synthesis exercise was primarily to develop a simple and direct route to these compounds, suitable for the preparation of sizable quantities for further spectroscopic studies and testing of biological activity. As such, stereocontrolled methods for the synthesis of the trisubstituted olefin moiety were not examined, particularly as this unit was readily available by well established protocols. The primary issue was the development of a reliable method for the synthesis of the 2-substituted tetrahydropyridine unit. Two disconnections to this unit were examined, one involving the formation of the C_2-C_7 bond to a preformed heterocycle, and the other based on a ketimine forming reaction. The convergency of the first approach made it particularly attractive as it offered the possibility of exceedingly short syntheses of these natural products as well as their analogs. The first route examined was based on the reported high yielding synthesis of cyclic ketimines by the reaction of alkyllithiums with *N*-TMS-lactams (**Scheme 1**).[8] This reaction involves the nucleophilic addition of an alkyllithium followed, presumably, by a Peterson-type elimination of trimethylsiloxide anion to yield the imine linkage. We planned to prepare the required alkyllithium from the corresponding iodide, readily synthesized from methyl isodehydroacetate by the reported method.[9] The alcohol (**4**) was protected as the THP ether and the bromide was transformed into the iodide (**5**). The conversion of iodide **5** to the corresponding alkyllithium proved difficult. The use of standard metal-halogen exchange procedures (BuLi or *tert*-BuLi) followed by addition of *N*-TMS-δ-valerolactam (**6**)[10] afforded only trace amount of the desired cyclic ketimine (**7**). The desired transformation was accomplished, albeit in low yield, by using lithium 4,4'-di-*tert*-butylbiphenylide (LDBB)[11] for the reductive lithiation of iodide (**5**). Thus, addition of *N*-TMS-δ-valerolactam (**6**) to the mixture of **5** and LDBB in THF at -78 °C and subsequent stirring at -40 °C and then at -15 °C gave the expected ketimine (**7**). The THP protecting group was successfully removed under neutral condition[12] to give

Scheme 1: Synthesis of Polonicumtoxin C (3)—Route 1[a]



4[9] **5** **7** **3** **6**[10]

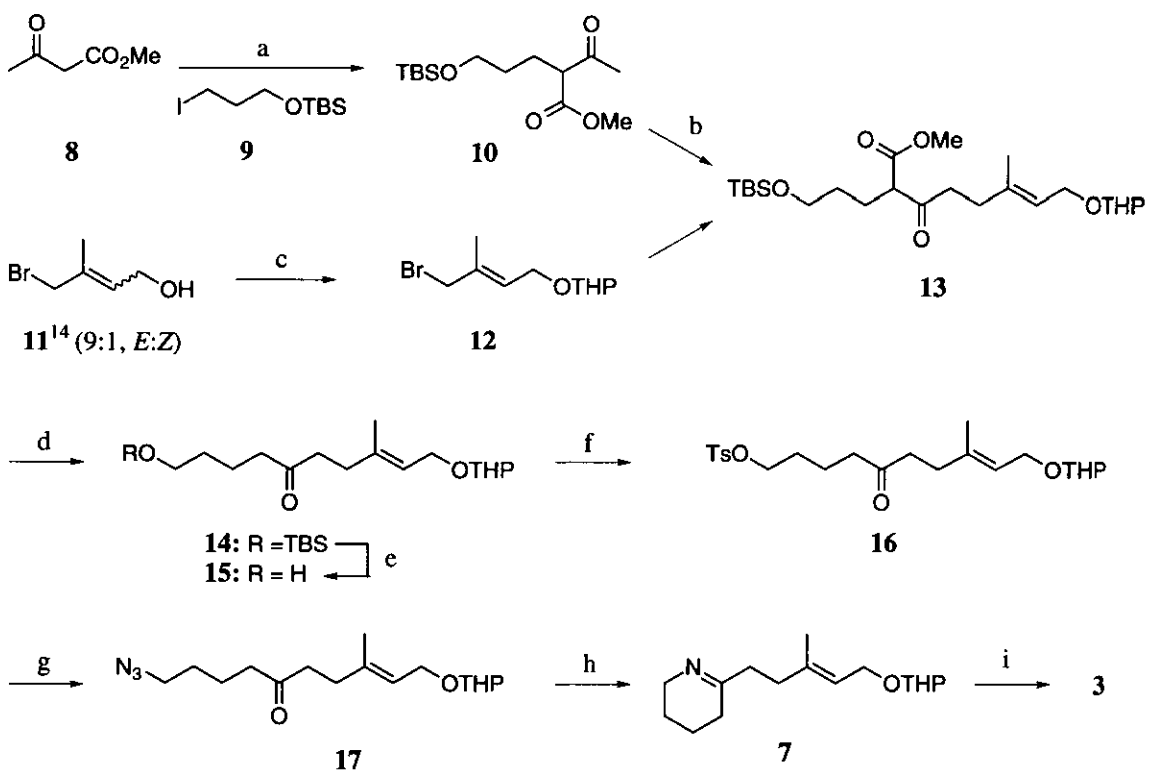
[a] Reagents and conditions: (a) DHP, cat. TsOH, CH_2Cl_2, 0 °C, 1 h; (b) NaI, acetone, reflux, 3 h (67% overall); (c) LDBB, THF, -78 °C, 15 min, then **6**, THF, -78°C, 30 min, -40 °C, 30 min, -15 °C, 1.5 h (10 %); (d) $MgBr_2$, ether, rt, 4 h (6% overall from **6**).

polonicumtoxin C (**3**). While the reaction sequence was short, the low yield for the key ketimine-forming coupling reaction, probably due to poor lithiation of the iodide (**5**), made this route unsatisfactory

Synthesis of Polonicumtoxin C (3) (Route 2)

The second route to the polonicumtoxins was based on the aza-Wittig reaction for construction of the ketimine unit.[13] The required keto azide precursor (**17**) was prepared in a straightforward manner as shown in **Scheme 2**. Methyl acetoacetate (**8**) was alkylated with the TBS-protected 3-iodo-1-propanol (**9**) to afford keto ester (**10**) in good yield. The olefin containing portion was also introduced by alkylation. Treatment of the dianion[14] of **10** with the *E* isomer of the bromide (**12**), prepared by THP protection of an *E*, *Z* mixture of the isoprene-derived bromohydrin (**11**)[15] followed by column chromatography separation, afforded keto ester (**13**), having the carbon skeleton required for the polonicumtoxins. Decarboxylation of **13** using methanolic sodium hydroxide gave the desired ketone (**14**), accompanied by a significant amount of the TBS-deprotected ketone (**15**) (**14**:**15** = 2:1). After

Scheme 2: Synthesis of Polonicumtoxin C (3)—Route 2[a]

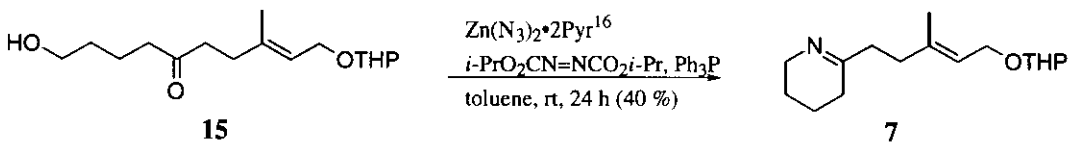


[a]Reagents and conditions: (a) NaH, **9**, THF, reflux, 16 h (66 %); (b) **10**, NaH, BuLi, THF, **12**, 0 °C, 30 min (65 %); (c) DHP, cat. TsOH, CH_2Cl_2, 0 °C, 30 min (81 %); (d) NaOH, MeOH, reflux, 30 min (**14**:**15**=2:1); (e) Bu_4NF, THF, rt, 2 h (52 % overall from **13**); (f) TsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 14 h (69 %); (g) NaN_3, DMF, rt, 7 h (90 %); (h) Ph_3P, ether, rt, 12 h (78 %); (i) $MgBr_2$, ether, rt, 12 h (60 %).

complete removal of TBS group using TBAF, the resulting hydroxyl group of **15** was converted sequentially to tosylate (**16**), and then to azide (**17**), precursor of the intramolecular aza-Wittig reaction. The pivotal ketimine formation was carried out under the Staudinger[16] reaction conditions. Stirring an ether solution of azide (**17**) and triphenylphosphine at room temperature cleanly produced expected imine (**7**) in good yield. The THP group was removed under the same condition as described in **Scheme 1** to afford the desired natural product (**3**).

Although lengthier than the first, the second route provided a workable synthesis of **3**. All the steps proceeded in good yield and were amenable to scale-up. Although we did not expend much effort in shortening this sequence, we did develop a useful one-pot procedure that eliminated two steps in the synthesis. It was clear that alcohol (**15**) could be converted to azide (**17**) by a one step procedure[17] rather than two by using a Mitsunobu-type substitution reaction. The Mitsunobu[18] reaction is carried out using triphenylphosphine, the same reagent needed for the next step, the Staudinger aza-Wittig reaction. It was expected that both of these processes could be carried out by simply using an excess of triphenylphosphine. Indeed, the desired cyclic ketimine (**7**) was formed directly on treatment of the alcohol with zinc azide, diisopropyl azodicarboxylate, and two equivalents of triphenylphosphine (**Scheme 3**). With this modification, the synthesis of **3** proceeded in only six steps from methyl acetoacetate.

Scheme 3: One Pot Preparation of 7 from 15

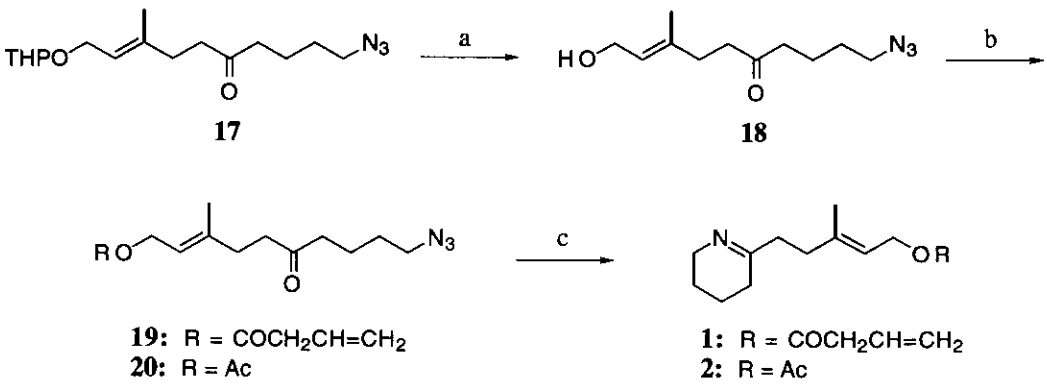


Synthesis of Polonicumtoxins A (1) and B (2).

The aza-Wittig protocol was also used for the synthesis of polonicumtoxins A (**1**) and B (**2**) (**Scheme 4**). The ester group present in these natural products was introduced after removal of the THP group in keto azide (**17**). The resulting azido keto esters (**19**) and (**20**) were subjected to the Staudinger reaction conditions to effect the aza-Wittig reaction and yield **1** and **2**, respectively.

The chemial shifts of ^{1}H and ^{13}C NMR signals and the coupling constants of the ^{1}H NMR signals of synthetic **1**, **2**, and **3** were identical to those of the natural products, thus unambiguously confirming the previously assigned structures. All these signals were assigned based on the ^{1}H-^{1}H COSY, ^{13}C decoupled HMQC and HMBC spectral data, measured using the synthetic samples (**Table 1**).

Scheme 4: Synthesis of Polonicumtoxins A (1) and B (2)[a]



[a]Reagents and conditions: (a) cat. PPTS, EtOH, 55 °C, 12 h (70 %); (b) for **19**: CH_2=$CHCH_2COOH$, DCC, DMAP, toluene, 0 °C, 15 min (73 %); for **20**: Ac_2O, pyr, DMAP, CH_2Cl_2, 0 °C, 2 h (74 %); (c) Ph_3P, ether, 15 °C, 12 h (**1**: 83 % from **19**, **2**: 79 % from **20**).

Table 1. NMR Data of Synthetic **1**, **2**, and **3** in CD_3CN[a]

position	**1** C(δ)[b]	H(δ)[c]	mult.	*J* (Hz)	**2** C(δ)[d]	H(δ)[e]	mult.	*J* (Hz)	**3** C(δ)[d]	H(δ)[e]	mult.	*J* (Hz)
2	169.8				170.5				170.6			
3	29.1	2.05 2H	tt	6.3, 1.8	29.5	2.11 2H	tt	6.3, 1.2	29.5	2.14 2H	tt	6.9, 1.2
4	20.2	1.58 1H	m		20.2	1.58 1H	m		20.1	1.59 1H	m	
		1.61 1H	m			1.61 1H	m			1.61 1H	m	
5	22.5	1.45 1H	m		22.5	1.48 1H	m		22.5	1.54 1H	m	
		1.48 1H	m			1.50 1H	m			1.56 1H	m	
6	49.4	3.40 2H	br t		49.4	3.40 2H	br t		49.3	3.40 2H	br t	
7	39.1	2.18 2H	br s		39.1	2.21 2H	br s		39.4	2.25 2H	t	7.9
8	36.3	2.18 2H	br s		36.3	2.21 2H	br s		36.4	2.16 2H	t	7.9
9	142.9				142.6				138.2			
10	119.2	5.29 1H	tq	6.3, 1.1	120.2	5.29 1H	tq	6.4, 1.1	125.3	5.28 1H	tq	6.7, 1.3
11	61.8	4.53 2H	d	6.3	61.5	4.49 2H	d	6.4	58.9	3.96 2H	d	6.7
12	16.3	1.66 3H	d	1.1	16.3	1.66 3H	br s		16.1	1.60 3H	br s	
1'	171.9				171.3							
2'	39.4	3.03 2H	dt	6.8, 1.4	20.9	1.94 3H	s					
3'	131.8	5.87 1H	m									
4'	118.3	5.08 1H	dq	10.9, 1.4								
		5.12 1H	dq	17.3, 1.4								

[a]CHD_2CN at 1.9 ppm was used as reference for ^{1}H NMR, and $CD_3{}^{13}CN$ at 118 ppm was used for ^{13}C NMR. [b]62.9 MHz. [c]300 MHz. [d]75 MHz. [e]400 MHz.

The *E* geometry assigned to the olefin in **1**, **2**, and **3** was confirmed by observing the positive NOE (1.4 %) at 11-CH_2 (3.96 ppm) when the 12-Me signal at 1.60 ppm was irradiated in the ^{1}H NMR spectrum of **3**. The ichthyotoxicities (LC_{99}) of synthetic **1**, **2**, and **3** to *O. latipes* were found to be 25,

50, 500 ppb, respectively, consistent with those observed for the naturally derived samples (13, 33, 300 ppb).[3]

In summary, the syntheses of polonicumtoxins A (**1**), B (**2**), and C (**3**), the first toxins to be isolated from fresh water dinoflagellates, were completed using an aza-Wittig reaction for the key cyclic ketimine-forming step. The synthetic samples were used to establish the structures and NMR assignments of the naturally derived toxins. The synthetic route developed here should allow the ready preparation of larger quantities of these ichthyotoxins and numerous analogs, for further pharmacological investigations.

EXPERIMENTAL

General Remarks. FT-IR spectra were measured as films using $CHCl_3$ as solvent on a Perkin-Elmer 1600 or Nicolet Impact 410. ^1H and ^{13}C NMR spectra were measured on a Brüker AM-200, 250, 300, JEOL GSX-400, or Varian Gemini 2000 spectrometers at 200, 250, 300 or 400 MHz for ^1H NMR and at 50, 63, or 75 MHz for ^{13}C NMR, respectively, using $CDCl_3$ or CD_3CN as the solvent. TMS was used as an internal standard when using $CDCl_3$. The signal of CHD_2CN at 1.9 ppm on ^1H NMR and the signal of CD_3CN at 118 ppm on ^{13}C NMR are used as the references when using CD_3CN. *J* values on ^1H NMR are given in Hz. On DEPT (135°) spectra, the signals of CH_3, CH_2, and CH are shown as positive (+), negative (-), and positive (+), respectively, and quaternary carbons are not shown. High-resolution and low-resolution EI MS spectra were obtained on a VG 70-250S or JEOL JMS303HF spectrometer. ESI (Electrospray ionization) MS were obtained on a Finnigan mat TSQ700. High-resolution FAB mass spectra were obtained on a JEOL JMS700 MS Station. Microanalyses were performed by the Microanalytical Section of Faculty of Pharmacology, Tohoku University. All reactions were performed under a positive pressure of argon, using freshly distilled solvents. Progress of the reactions was monitored using thin layer chromatography. Flash column chromatography was conducted on silica gel 60 (40-63 micron, unless otherwise specified).

***(E)*-5-Iodo-3-methyl-1-(tetrahydro-2-pyranyloxy)-2-pentene (5).** To a solution of (*E*)-5-bromo-3-methyl-2-penten-1-ol (**4**)[9] (287 mg, 1.6 mmol) in 5 mL of CH_2Cl_2 was added 3,4-dihydro-2*H*-pyran (DHP, 202 mg, 2.4 mmol) and *p*-toluenesulfonic acid (TsOH, 10 mg, 0.05 mmol) at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was diluted with ether (15 mL), neutralized with saturated aqueous $NaHCO_3$ (15 mL), and extracted with ether (3x15 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. To a solution of this crude THP ether in 3 mL of acetone was added NaI (1.2 g, 7.8 mmol). After refluxing for 3 h, the reaction mixture was diluted with 15 mL of ether, treated with saturated aqueous $Na_2S_2O_3$ (15 mL), and extracted with ether (3x15 mL). The combined organic extracts were washed with brine

(30 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 5:1), affording **5** (331 mg, 67 % overall yield) as an oil. IR 2940, 2869, 2849, 1452, 1440, 1353, 1199, 1131, 1117, 1075, 1023, 963 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.62 (br s, 3H, CH_3-6), 1.48-1.81 (m, 6H, THP $(CH_2)_3$), 2.53 (t, 2H, H-4, *J*=8), 3.18 (t, 2H, H-5, *J*=8), 3.47 (m, 1H, THP C*H*HO), 3.82 (m, 1H, THP CH*H*O), 3.98 (dd, 1H, C*H*H-1, *J*=13, 7), 4.17 (dd, 1H, CH*H*-1, *J*=13, 7), 4.58 (t, 1H, THP acetal H, *J*=3), 5.35 (tq, 1H, H-2, *J*=7, 1); ^{13}C NMR (75 MHz, $CDCl_3$), δ 3.4 (-, C5), 15.5 (+, C6), 19.4 (-, THP CH_2), 25.2 (-, THP CH_2), 30.4 (-, THP CH_2), 43.4 (-, C4), 62.1 (-, THP CH_2O), 63.1 (-, C1), 97.6 (+, THP acetal), 123.4 (+, C2), 138.0 (C3); EIMS *m/z* 310 (M^+, 1), 254 (M^+ -C_4H_8, 37), 226 (M^+ -C_6H_{12}, 8), 209 (M^+-OTHP, 14), 127 (I, 50), 85 (THP, 88), 81 ($C_2H_4CCH_3CHCH$, 100); HREIMS calcd for $C_{11}H_{19}O_2I$ 310.0432, found 310.0437.

Polonicumtoxin C (3) (Route 1). Lithium 4,4'-di-*tert*-buthylbiphenylide (LDBB) was prepared as follows.[11] Lithium wire (21 mg, 3 mmol), cut into several pieces and washed with hexanes, was added to a flame dried flask containing 4,4'-di-*tert*-buthylbiphenyl (DBB, 399 mg, 1.5 mmol) in THF (2 mL). The mixture was sonicated at 0 °C for 15 min, and then stirred at the same temperature for 4 h. To the resulting solution of LDBB cooled to -78 °C was added dropwise a solution of **5** (95.2 mg, 0.3 mmol) in THF (0.5 mL). After stirring at -78 °C for 15 min, a solution of *N*-TMS-δ-valerolactam (**6**,[10] 52 mg, 0.3 mmol) in THF (0.5 mL) was added dropwise. After 30 min, the reaction mixture was allowed to warm to -40 °C and stirred for 30 min, then warmed to -15 °C and stirring was continued for 1.5 h. The reaction was quenched with a few drops of saturated aqueous NH_4Cl at 0 °C. After 10 min, the reaction mixture was diluted with ether (15 mL), neutralized with a further portion of saturated aqueous NH_4Cl (15 mL), and extracted with ether (3x15 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting crude product was subjected to flash chromatography (10x40 mm) using CH_2Cl_2. After eluting with 20 mL of CH_2Cl_2, the solvent was changed to CH_2Cl_2-MeOH (9:1, 20 mL), which allowed the elution of **7** and δ-valerolactam as a mixture in a 1:9 ratio as determined by ^1H NMR. According to this ratio, the yield of **7** was estimated to be approximately 10 %. The THP group of **7** was deprotected without further purification as follows. To a solution of the mixture (60 mg) of **7** and δ-valerolactam in ether (2 mL) was added $MgBr_2$ (22 mg, 0.12 mmol) at rt (15 °C). After stirring for 4 h, the solvent was removed by evaporation, and the residue was extracted with CH_2Cl_2 (3x15 mL). The bombined extracts were concentrated, and the crude oil was purified by flash chromatography. The column was sequentially washed with CH_2Cl_2 (20 mL), CH_2Cl_2-MeOH (9:1, 20 mL), and with CH_2Cl_2-MeOH (7:3, 20 mL), which resulted in the elution of pure **3** as an oil (3.2 mg, 6% overall yield from **6**,). Spectral data of **7** are shown below (see **7** and **3** by Route 2) and those of **3** are shown in Table 1.

3-Iodo-1-*tert*-butyldimethylsiloxypropan (9). A solution of 3-bromopropan-1-ol (1.42 g, 10.2 mmol) in CH_2Cl_2 (50 mL) was treated with imidazole (1.04 g, 15.3 mmol), and a solution of *tert*-butyldimethylsilyl chloride (TBSCl, 1.69 g, 11.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise at 0 °C over 5 min. After 5 min of stirring at 0 °C, the mixture was allowed to warm to rt (15 °C). After 5 h, the reaction mixture was diluted with ether (30 mL) and neutralized with a saturated aqueous NH_4Cl (30 mL). The organic phase was separated and the aqueous phase was extracted with ether (3x30 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash chromatography (hexanes/EtOAc, 9:1) provided 3-bromo-1-*tert*-butyldimethylsiloxypropan (2.44 g, 9.49 mmol, 98 % yield as oil), which was used directly for the next step.

To a solution of 3-bromo-1-*tert*-butyldimethylsiloxypropan (2.44 g, 9.49 mmol) in acetone (25 mL) was added NaI (14.2 g, 94.9 mmol). After refluxing for 1.5 h, the reaction mixture was diluted with 50 mL of ether, treated with saturated aqueous $Na_2S_2O_3$ (25 mL), amd extracted with ether (3x25 mL). The combined organic extracts were washed with brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 9:1 then 5:1), affording **9** (2.53 g, 87 % overall yield from 3-bromopropan-1-ol) as a waxy white solid. IR 2954, 2927, 2856, 1471, 1256, 1182, 1100, 1052, 1006, 931, 834, 776 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$), δ 0.08 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 2.00 (m, 2H, CH_2-2), 3.29 (t, 2H, CH_2-3, *J*=7), 3.68 (t, 2H, CH_2-1, *J*=5); ^{13}C NMR (50 MHz, $CDCl_3$), δ -5.3 (+, $Si(CH_3)_2$), 3.5 (-, C3), 18.3 ($C(CH_3)_3$), 25.9 (+, $C(CH_3)_3$), 36.2 (-, C2), 62.4 (-, C1).

Methyl 2-acetyl-5-*tert*-butyldimethylsiloxypentanoate (10). NaH (60 % dispersion in mineral oil, 44 mg, 1.1 mmol) was washed with hexanes and dried under Ar. To a suspension of NaH in THF (1 mL) was added dropwise a solution of methyl acetoacetate (**8**) (116 mg, 1.0 mmol) in THF (1 mL) at 0 °C. After 5 min of stirring, a solution of **9** (306 mg, 1.0 mmol) in THF (1 mL) was added dropwise, and the resulting mixture was refluxed for 15.5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (15 mL) at 0 °C, and then diluted with ether (15 mL). After extraction with ether (3x15 mL), the combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 9:1 and then 3:1), affording **10** (225 mg, 66 %) as an oil. IR 2954, 2858, 1744, 1719, 1472, 1437, 1360, 1256, 1219, 1153, 1095, 1006, 835, 777 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$), δ 0.08 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 1.55 (m, 2H, CH_2-4), 1.96 (q, 2H, CH_2-3, *J*=7), 2.26 (s, 3H, CH_3CO), 3.54 (t, 1H, H-2, *J*=7), 3.66 (t, 2H, CH_2-5, *J*=5), 3.77 (s, 3H, OCH_3); ^{13}C NMR (50 MHz, $CDCl_3$), δ -5.4 (+, $Si(CH_3)_2$), 18.1 ($C(CH_3)_3$), 24.7 (+, CH_3CO), 25.8 (+, $C(CH_3)_3$), 28.6 (-, C3), 30.2 (-, C4), 52.1 (+, C2), 59.3 (+, OCH_3), 62.3 (-, C5), 170.1 (C1),

202.9 (CH_3CO); ESIMS *m/z* 289 [$(M+H)^+$, 28], 311 [$(M+Na)^+$, 100], 327 [$(M+K)^+$, 52]; HRFABMS calcd for $C_{14}H_{28}O_4NaSi$ 311.1655, found 311.1603.

(*E*)-4-Bromo-3-methyl-1-(tetrahydro-2-pyranyloxy)-2-buten (12). To a solution of *E, Z* mixture of 4-bromo-3-methyl-2-buten-1-ol (**11**)[15] (*E /Z*, 9:1, 462 mg, 2.80 mmol) in CH_2Cl_2 (30 mL) was added DHP (283 mg, 3.36 mmol) and TsOH (10 mg, 0.058 mmol) at 0 °C. After 30 min of stirring, the reaction was quenched with saturated aqueous $NaHCO_3$ (35 mL), and the mixture was extracted with ether (3x30 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography (hexanes/EtOAc, 20:1) allowed separation of the *E* and *Z* isomers, providing **12** as an oil (508 mg, 81 %). IR 2941, 2360, 1440, 1354, 1200, 1118, 1076, 1024, 906, 869, 814 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$), δ 1.48-1.91 (m, 6H, THP $(CH_2)_3$), 1.82 (d, 3H, CH_3-5, *J*=1), 3.55 (m, 1H, THP C*H*HO), 3.87 (m, 1H, THP CH*H*O), 3.98 (s, 2H, H-4), 4.04 (dd, 1H, C*H*H-1, *J*=13, 6), 4.26 (dd, 1H, CH*H*-1, *J*=13,6), 4.62 (t, 1H, THP acetal H, *J*=3), 5.78 (tq, 1H, H-2, *J*=6,1); ^{13}C NMR (50 MHz, $CDCl_3$), δ 14.9 (+, C5), 19.3 (-, THP CH_2), 25.3 (-, THP CH_2), 30.4 (-, THP CH_2), 40.1 (-, C4), 62.0 (-, THP CH_2O), 63.3 (-, C1), 97.9 (+, THP acetal), 126.9 (+, C2), 135.1 (C3); ESIMS *m/z* 271, 273 [$(M+Na)^+$, 26, 26]; Anal. Calcd for $C_{10}H_{17}O_2Br$: C, 48.19; H, 6.83; Br, 32.13, Found: C, 48.72, H, 6.85, Br, 32.35.

***(E)*-Methyl 2-(3-*tert*-butyldimetylsiloxypropyl)-6-methyl-3-oxo-8-(tetrahydro-2-pyranyloxy)-6-octenoate (13).** NaH (60 % dispersion in mineral oil, 306.8 mg, 7.67 mmol) was treated as described above (see the preparation of **10**). To a suspension of oil-free NaH in THF (10 mL) was added dropwise a solution of **10** (1.69 g, 5.9 mmol) in THF (15 mL) at 0 °C over 5 min, and the resultant mixture was stirred for 10 min. A solution of BuLi (3.3 mL, 2.3 M in hexane, 7.67 mmol) was then added dropwise to the mixture at 0 °C over 5 min. After 10 min, a solution of **12** (1.47 g, 5.9 mmol) in THF (15 mL) was added dropwise over 5 min. After stirring at 0 °C for 30 min, the reaction was quenched with a few drops of saturated aqueous NH_4Cl, and the mixture was partitioned between ether (25 mL) and saturated aqueous NH_4Cl (25 mL). The organic phase was separated, and the aqueous phase was extracted with ether (3x50 mL). The combined organic extracts were washed with brine (2x25 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting crude oil was purified by flash chromatography (hexanes/EtOAc, 9:1 and then 3:1), affording **13** (1.77 g, 65 %) as an oil. IR 2953, 2857, 1747, 1718, 1463, 1440, 1360, 1256, 1201, 1164, 1101, 1023, 888, 777 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$), δ 0.08 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 1.48-1.91 (m, 6H, THP $(CH_2)_3$), 1.55 (m, 2H, propyl CH_2-2'), 1.67 (br s, 3H, CH_3-9), 1.92 (q, 2H, propyl CH_2-1', J=7), 2.31 (t, 2H, CH_2-5, *J*=8), 2.64 (t, 2H, CH_2-4, *J*=8), 3.52 (t, 1H, H-2, *J*=7), 3.58 (m, 1H, THP C*H*HO), 3.61 (t, 2H, propyl CH_2-3', *J*=6), 3.72 (s, 3H, OCH_3), 3.89 (m, 1H,

THP CH*H*O), 4.00 (dd, 1H, C*H*H-8, *J*=13, 6), 4.23 (dd, 1H, CH*H*-8, *J*=13, 6), 4.61 (t, 1H, THP acetal H, *J*=3), 5.35 (br t, 1H, H-7, *J*=6); ^{13}C NMR (63 MHz, $CDCl_3$) δ -5.4 (+, $Si(CH_3)_2$), 16.5 (+, C9), 18.2 ($C(CH_3)_3$), 19.5 (-, THP CH_2), 24.8 (-, propyl C1'), 25.4 (-, THP CH_2), 25.9 (+, $C(CH_3)_3$), 30.3 (- , propyl C2'), 30.6 (-, THP CH_2), 32.7 (-, C5), 40.2 (-, C4), 52.2 (+, C2), 58.6 (+, OCH_3), 62.2 (-, THP CH_2O), 62.5 (-, propyl C3'), 63.3 (-, C8), 98.0 (+, THP acetal), 121.3 (+, C7), 138.2 (C6), 170.1 (C1), 204.5 (C3); ESIMS *m/z* 479 [(M+Na)$^+$, 100]; HRFABMS calcd for $C_{24}H_{44}O_6NaSi$ 479.2805, found 479.2792.

***(E)*-1-Hydroxy-8-methyl-10-(tetrahydro-2-pyranyloxy)-8-decen-5-one (15).** A solution of **13** (800 mg, 1.7 mmol) in MeOH (40 mL) was treated with NaOH (solid, 3.2 g, 80 mmol), and the mixture was heated to reflux for 30 min. The mixture was concentrated in vacuo to remove the solvent, and the residue was suspended with ether (35 mL), neutralized with saturated aqueous NH_4Cl (50 mL), and extracted with ether (3x50 mL) and CH_2Cl_2 (2x25 mL). The insoluble precipitates in the organic phase were removed by filtration. The combined organic extracts were washed with brine (2x50 mL), dried over Na_2SO_4, and concentrated in vacuo. The ^{1}H NMR of the crude mixture indicated the presence of two products, identified as **14** and its desilylated product **15** in a 2:1 ratio. For the complete cleavage of the TBS group of **14**, 1M solution of Bu_4NF in THF (3 mL) was added to the crude mixture of **14** and **15** in THF (67 mL) at rt (15 °C), and the mixture was stirred at rt for 2 h. The reaction mixture was diluted with ether (25 mL), washed with brine (25 mL), and extracted with ether (3x25 mL) and then CH_2Cl_2 (2x25 mL). The combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 3:1 and then EtOAc), affording **15** (258 mg, 52 % overall for the two steps) as an oil. IR 3452, 2940, 2870, 1711, 1441, 1380, 1200, 1116, 1076, 1022, 905, 868, 813 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$), δ 1.48-1.91 (m, 10H, THP $(CH_2)_3$, CH_2-2, CH_2-3), 1.68 (br s, 3H, CH_3-11), 2.30 (t, 2H, CH_2-7, *J*=8), 2.46 (t, 2H, CH_2-4, *J*=6), 2.55 (t, 2H, CH_2-6, *J*=8), 3.51 (m, 1H, THP C*H*HO), 3.62 (t, 2H, CH_2-1, *J*=6), 3.88 (m, 1H, THP CH*H*O), 4.00 (dd, 1H, C*H*H-10, *J*=13, 8), 4.22 (dd, 1H, CH*H*-10, *J*=13, 8), 4.60 (t, 1H, THP acetal H, *J*=3), 5.34 (br t, 1H, H-9, *J*=8); ^{13}C NMR (63 MHz, $CDCl_3$), δ 16.5 (+, C11), 19.6 (-, THP CH_2), 19.7 (-, C3), 25.4 (-, THP CH_2), 30.7 (-, THP CH_2), 32.1 (-, C2), 33.2 (-, C7), 40.9 (-, C4), 42.3 (-, C6), 62.2 (-, THP CH_2O), 62.3 (-, C1), 63.5 (-, C10), 98.0 (+, THP acetal), 121.0 (+, C9), 146.0 (C8), 210.4 (C5); ESIMS *m/z* 307 [(M+Na)$^+$, 20]; HRFABMS calcd for $C_{16}H_{28}O_4Na$ 307.1885, found 307.1828.

***(E)*-8-Methyl-10-(tetrahydro-2-pyranyloxy)-1-(*p*-toluenesulfonyloxy)-8-decen-5-one (16).** To a solution of **15** (110 mg, 0.41 mmol) in CH_2Cl_2 (15 mL) at 0 ℃ was added sequentially triethylamine (TEA, 228.6 μL, 1.64 mmol) and *p*-toluenesulfonyl chloride (TsCl, 187 mg, 0.984

mmol). The mixture was gradually warmed to rt (15 °C) over 1 h. After stirring at rt for 14 h, the reaction mixture was diluted with ether (25 mL), neutralized with saturated aqueous NH_4Cl (25 mL), and extracted with ether (3x50 mL) and then CH_2Cl_2 (2x25 mL). The combined organic layer was washed with brine (25 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 3:1 and then 1:1), affording **16** (120.5 mg, 69 %) as an oil. IR 2924, 1712, 1454, 1363, 1176, 1022, 749 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$), δ 1.48-1.91 (m, 10H, THP $(CH_2)_3$, CH_2-2, CH_2-3), 1.68 (d, 3H, CH_3-11, *J*=1), 2.28 (t, 2H, CH_2-7, *J*=8), 2.40 (t, 2H, CH_2-4, *J*=8), 2.45 (s, 3H, arom CH_3), 2.51 (t, 2H, CH_2-6, *J*=8), 3.55 (m, 1H, THP C*H*HO), 3.88 (m, 1H, THP CH*H*O), 4.02 (s, 2H, CH_2-1), 4.02 (dd, 1H, C*H*H-10, *J*=12, 6), 4.22 (dd, 1H, CH*H*-10, *J*=12, 6), 4.60 (t, 1H, THP acetal H, *J*=4), 5.33 (tq, 1H, H-9, *J*=6, 1), 7.34 (d, 2H, arom, *J*=8), 7.78 (d, 2H, arom, *J*=8); ^{13}C NMR (63 MHz, $CDCl_3$), δ 16.5 (+, C11), 19.6 (-, THP CH_2,), 19.6 (-, C3), 21.6 (+, arom CH_3), 25.4 (-, THP CH_2), 28.2 (-, C2), 30.7 (-, THP CH_2), 33.1 (-, C7), 41.0 (-, C4), 41.6 (-, C6), 62.3 (-, THP CH_2O), 63.5 (-, C10), 70.1 (-, C1), 98.0 (+, THP acetal), 121.2 (+, C9), 127.8 (+, arom CSO_3CH), 129.8 (+, arom $C(CH_3)CH$), 133.1 (arom CCH_3), 138.6 (C8), 144.7 (arom CSO_3), 209.4 (C5); ESIMS *m/z* 461 [$(M+Na)^+$, 36]; HRFABMS calcd for $C_{23}H_{35}O_6S$ 439.2154, found 439.2244.

***(E)*-1-Azido-8-methyl-10-(tetrahydro-2-pyranyloxy)-8-decen-5-one (17).** Sodium azide (268 mg, 4.2 mmol) was added to a solution of **16** (120 mg, 0.28 mmol) in DMF (5 mL) at rt (15 °C). After stirring at rt for 7 h, 5 mL of water was added, and the mixture was extracted with ether (3x10 mL) and then CH_2Cl_2 (2x10 mL). The combined organic phase was dried over Na_2SO_4 and concentrated in vacuo. Purification of the crude product by flash chromatography (hexanes/diethylamine (DEA), 9:1), afforded **17** (77.9 mg, 90 %) as an oil. IR 2922, 2851, 2096, 1742, 1718, 1458, 1372, 1239, 1048, 906, 749 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.48-1.91 (m, 10H, THP $(CH_2)_3$, CH_2-2, CH_2-3), 1.68 (d, 3H, CH_3-11, *J*=1), 2.30 (t, 2H, CH_2-7, *J*=8), 2.45 (t, 2H, CH_2-4, *J*=6), 2.54 (t, 2H, CH_2-6, *J*=8), 3.28 (t, 2H, CH_2-1, *J*=7), 3.50 (m, 1H, THP C*H*HO), 3.88 (m, 1H, THP CH*H*O), 4.01 (dd, 1H, C*H*H-10, *J*=13, 7), 4.23 (dd, 1H, CH*H*-10, *J*=13, 7), 4.61 (t, 1H, THP acetal H, *J*=3), 5.35 (tq, 1H, H-9, *J*=7, 1); ^{13}C NMR (63 MHz, $CDCl_3$), δ 16.6 (+, C11), 19.6 (-, THP CH_2), 20.8 (-, C3), 25.4 (-, THP CH_2), 30.7 (-, THP CH_2), 28.4 (-, C2), 33.2 (-, C7), 41.0 (-, C4), 42.0 (-, C6), 62.3 (-, THP CH_2O), 51.2 (-, C1), 63.5 (-, C10), 98.0 (+, THP acetal), 121.0 (+, C9), 138.6 (C8), 209.6 (C5); ESIMS *m/z* 332 [$(M+Na)^+$, 100], 348 [$(M+K)^+$, 74]; HRFABMS calcd for $C_{16}H_{27}N_3O_3Na$ 332.1950, found 332.1893.

11-*O*-(Tetrahydro-2-pyranyl) polonicumtoxin C (7) (Route 2). Triphenylphosphine (48 mg, 0.183 mmol) was added to a solution of **17** (18.7 mg, 0.061 mmol) in ether (2 mL) at rt (15 °C).

After stirring for 12 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (2 mL), and the mixture was extracted with ether (3x5 mL) and then with CH_2Cl_2 (2x5 mL). The combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The crude oil was charged onto a flash chromatography column with hexanes/EtOAc (3:1), and eluted with hexanes/DEA (3:1) to give **7** (oil, 10.7 mg, 78 %). IR 2924, 2855, 2360, 1745, 1456, 1373, 1239, 1048, 1024 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.48-1.91 (m, 10H, THP $(CH_2)_3$, CH_2-4, CH_2-5), 1.69 (br s, 3H CH_3-12), 2.11 (tt, 2H, CH_2-3, *J*=7, 2), 2.26 (br s, 4H, CH_2-7, CH_2-8), 3.48 (m, 1H, THP C*H*HO), 3.56 (br t, 2H, CH_2-6, *J*=5), 3.87 (m, 1H, THP CH*H*O), 4.23 (dd, 1H, C*H*H-11, *J*=13, 6), 4.30 (dd, 1H, CH*H*-11, *J*=13, 6), 4.61 (t, 1H, THP acetal H, *J*=3), 5.35 (br t, 1H, H-10, *J*=6); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.4 (+, C12), 19.5 (-, C5), 19.6 (-, THP CH_2), 21.9 (-, C4), 23.9 (-, C3), 25.4 (-, THP CH_2), 30.7 (-, THP CH_2), 36.1 (-, C8), 39.1 (-, C7), 49.2 (-, C6), 62.3 (-, THP CH_2O), 63.5 (-, C11), 98.0 (+, THP acetal), 121.9 (+, C10), 139.7 (C9), 170.3 (C2); ESIMS *m/z* 307 [$(M+Na)^+$, 20]. EIMS *m/z* 265 (M^+, 3), 164 [$(M-O-THP)^+$, 100]; HREIMS calcd for $C_{16}H_{27}NO_2$ 265.2064, found 265.2035.

11-*O*-(Tetrahydro-2-pyranyl) polonicumtoxin C (7) (one pot preparation from 15). To a solution of **15** (6.1 mg, 0.021 mmol) in toluene (1 mL) was added ZnN_6•2Pyr[16] (9.6 mg, 0.032 mmol), di-*iso*-propyl azodicarboxylate (8.46 mg, 0.042 mmol), and triphenylphosphine (11 mg, 0.042 mmol) at rt (15 °C). After stirring for 20 h, the reaction was quenched with water (2 mL), and the mixture was extracted with ether (3x5 mL) . The combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The crude oil was placed on a flash chromatography column with EtOAc, and the column was sequentially eluted with EtOAc, CH_2Cl_2 and CH_2Cl_2-MeOH (9:1), which afforded pure **7** (2.1 mg, 40 %) as an oil.

Polonicumtoxin C (3) (Route 2). To a solution of **7** (23.8 mg, 0.093 mmol) in ether (2 mL) was added $MgBr_2$ (99.4 mg, 0.46 mmol) at rt (15 °C), and the mixture was stirred for 12 h. From this reaction mixture, **3** was purified as described above (see: Route 1) (10.1 mg, 60%). IR 3354, 2933, 2872, 2359, 2226, 1656, 1454 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 181 (M^+, 5), 164 [$(M-OH)^+$, 45], 150 [$(M-CH_2OH)^+$, 100]; HREIMS calcd for $C_{11}H_{19}NO$ 181.1467, found 181.1470.

***(E)*-1-Azido-10-hydroxy-8-methyl-8-decen-5-one (18).** A catalytic amount of pyridinium *p*-toluenesulfonate (PPTS, 22.6 mg, 0.09 mmol) was added to a solution of **17** (56 mg, 0.181 mmol) in EtOH (4 mL), and the resulting solution was stirred at 55 °C for 12 h. The reaction was quenched with saturated aqueous NH_4Cl (5 mL), and the mixture was extracted with ether (3x15 mL). The combined organic extract was washed with brine (5 mL), dried over Na_2SO_4, and concentrated in

vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 3:1 and then 1:1), affording **18** (28.7 mg, 70 %) as an oil. IR 3406, 2931, 2360, 2341, 2096, 1711, 1446, 1410, 1377, 1269, 1113, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 1.54-1.71 (m, 4H, CH_2-2, CH_2-3), 1.68 (br s, 3H, CH_3-11), 2.30 (t, 2H, CH_2-7, *J*=8), 2.46 (t, 2H, CH_2-4, *J*=7), 2.55 (t, 2H, CH_2-6, *J*=8), 3.28 (t, 2H, CH_2-1, *J*=7), 4.15 (d, 2H, CH_2-10, *J*=7), 5.39 (br t, 1H, H-9, *J*=7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.4 (+, C11), 20.8 (-, C3), 28.3 (-, C2), 33.1 (-, C7), 40.9 (-, C4), 42.0 (-, C6), 51.2 (-, C1), 59.2 (-, C10), 123.8 (+, C9), 138.1 (C8), 209.5 (C5); ESIMS *m/z* 248 [$(M+Na)^+$, 100], 264 [$(M+K)^+$, 14]; HRFABMS calcd for $C_{11}H_{19}N_3O_2Na$ 248.1375, found 248.1369.

***(E)*-1-Azido-10-(3-butenoyloxy)-8-methyl-8-decen-5-one (19).** To a solution of **18** (9 mg, 0.04 mmol) in toluene (0.5 mL) was added dicyclohexylcarbodiimide (DCC, 20.6 mg, 0.1 mmol), DMAP (2.2 mg, 0.018 mmol) and 3-butenoic acid (3.4 mg, 0.04 mmol) at 0 °C. The mixture was allowed to warm to rt (15 °C) over 15 min, and the mixture was stirred for further 15 min. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) and the mixture was extracted with ether (3x5 mL). The combined organic extract was washed with brine (5 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 9:1 and then 3:1), yielding **19** (8.5 mg, 73 %) as an oil. IR 2932, 2349, 2096, 1736, 1713, 1446, 1261, 1167 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$), δ 1.54-1.71 (m, 4H, CH_2-2, CH_2-3), 1.71 (d, 3H, CH_3-11, *J*=1), 2.31 (t, 2H, CH_2-7, *J*=8), 2.45 (t, 2H, CH_2-4, *J*=7), 2.54 (t, 2H, CH_2-6, *J*=8), 3.08 (dt, 2H, butenoate CH_2 CO, *J*=7, 2), 3.28 (t, 2H, CH_2-1, *J*=6), 4.59 (d, 2H, CH_2-10, *J*=7), 5.16 (dq, 1H, C*H*H=CH, *J*=17, 2), 5.17 (dq, 1H, CH*H*=CH, *J*=10, 2), 5.34 (tq, 1H, H-9, *J*=7, 1), 5.92 (ddt, 1H, CH_2=C*H*, *J*=17, 10, 7); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.6 (+, C11), 20.8 (-, C3), 28.3 (-, C2), 33.1 (-, C7), 39.1 (butenoate CH_2 CO), 40.8 (-, C4), 42.0 (-, C6), 51.2 (-, C1), 61.4 (-, C10), 118.4 (-, CH_2=CH), 118.8 (+, C9), 131.8 (+, CH_2=CH), 140.8 (C8), 171.5 (COO), 209.3 (C5); ESIMS *m/z* 316 [$(M+Na)^+$, 100], 322 [$(M+K)^+$, 22]; HRFABMS calcd for $C_{15}H_{23}N_3O_3Na$ 316.1637, found 316.1676.

***(E)*-1-Azido-10-acetoxy-8-methyl-8-decen-5-one (20).** To a solution of **18** (15.7 mg, 0.070 mmol) in CH_2Cl_2 (1 mL) was added pyridine (26.5 mg, 0.336 mmol), DMAP (0.86 mg, 0.0070 mmol), and acetic anhydride (17.2 mg, 0.17 mmol) at 0 °C. After 2 h of stirring at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl (2 mL), and the mixture was extracted with ether (3x5 mL). The combined organic extracts were washed with brine (5 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 9:1 and then 3:1), affording **20** (13.9 mg, 74 %) as an oil. IR 2919, 2848, 2095, 1737, 1460, 1371, 1233, 1024, 747 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$), δ 1.54-1.71 (m, 4H, CH_2-2, CH_2-3), 1.71 (d, 3H,

CH_3-11, *J*=1), 2.04 (s, 3H, CH_3CO), 2.31 (t, 2H, CH_2-7, *J*=8), 2.46 (t, 2H, CH_2-4, *J*=7), 2.54 (t, 2H, CH_2-6, *J*=8), 3.28 (t, 2H, CH_2-1, *J*=7), 4.57 (d, 2H, CH_2-10, *J*=7), 5.33 (tq, 1H, H-9, *J*=7, 1); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.5 (+, C11), 20.8 (+, CH_3CO), 20.8 (-, C3), 28.3 (-, C2), 33.0 (-, C7), 40.8 (-, C4), 42.0 (-, C6), 51.2 (-, C1), 61.1 (-, C10), 118.9 (+, C9), 140.6 (C8), 171.0 (*C*OO), 209.6 (C5); ESIMS *m/z* 290 [$(M+H)^+$, 32]; HRFABMS calcd for $C_{13}H_{21}N_3O_3Na$ 290.1481, found 290.1516.

Polonicumtoxin A (1). Triphenylphosphine (48.8 mg, 0.19 mmol) was added to a solution of **19** (18.2 mg, 0.062 mmol) in ether (1 mL) at rt (15 °C). After stirring for 12 h, the reaction was quenched with 2 mL of saturated aqueous $NaHCO_3$ (2 mL). The mixture was extracted with ether (3x5 mL), the combined organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The resulting crude oil was purified by flash chromatography (hexane and then hexanes/DEA, 95:5), affording **1** (12.8 mg, 83 %) as an oil . IR 2926, 2862, 2360, 1736, 1661, 1445, 1375, 1245, 1171, 1102, 1047, 974, 919 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 249 (M^+, 1), 164 [$(M-OCOCH_2CHCH_2)^+$, 100]; HREIMS calcd for $C_{15}H_{23}NO_2$ 249.1729, found 249.1730.

Polonicumtoxin B (2). A solution of **20** (25.5 mg, 0.043 mmol) in ether (1 mL) was treated with triphenylphosphine (75.5 mg, 0.29 mmol) at rt (15 °C). After stirring for 12 h, the reaction was quenched with 2 mL of saturated aqueous $NaHCO_3$ (2 mL), and then extracted with ether (3x5 mL) . The combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The crude oil was purified by flash chromatography (hexanes/EtOAc, 3:1 and then hexanes/DEA, 9:1), affording **2** (17 mg, 79 %) as an oil. IR 2932, 1738, 1660, 1435, 1369, 1232, 1021, 949 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 223 (M^+, 1), 164 [$(M-OAc)^+$, 100]; HREIMS calcd for $C_{13}H_{21}NO_2$ 223.1572, found 223.1583

Bioassay. Three killifish *Oryzias latipes* were placed in 50 mL of test solution prepared by dissolving the sample in 0.04 M Tris-HCl buffer (pH 8.8).[3] The toxicities are expressed in LC_{99}, the minimum lethal concentration which kills three fish within 30-40 min.

ACKNOWLEDGMENTS

We thank Dr. Seiji Iwasa of Ohio State University (present address: Tokyo Institute of Technology) and Dr. Yasukatsu Oshima of Tohoku University for valuable discussion on this work.

This work was supported by grant-in-aid from the Ministry of Education, Science, Sports and Culture, Japan (No. 07102002), a SUNBOR grant, and grants from the Naito Foundation.

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Received, 22nd September, 1997