

Total Synthesis of Pectenotoxin-2**

Kenshu Fujiwara,* Yuki Suzuki, Nao Koseki, Yu-ichi Aki, Yuta Kikuchi, Shun-ichi Murata, Fuyuki Yamamoto, Mariko Kawamura, Toshio Norikura, Hajime Matsue, Akio Murai, Ryo Katoono, Hidetoshi Kawai, and Takanori Suzuki

Abstract: Pectenotoxin-2 (PTX2) is a shellfish toxin and has a non-anomeric spiroacetal, which is not stabilized by an anomeric effect. The selective construction of the non-anomeric spiroacetal has been a major problem in the synthesis of PTX2. Described herein is the stereoselective total synthesis of PTX2 via the isomerization of anomeric spiroacetal pectenotoxin-2b (PTX2b). The synthesis of PTX2b was achieved by a simple process including sulfone-mediated assembly of spirocyclic and bicyclic acetals and subsequent macrocyclization by ring-closing olefin metathesis. Finally, the selective construction of PTX2 was accomplished by the early termination of a dynamic transition process to equilibrium in the acid-catalyzed isomerization of anomeric PTX2b. [6,6]-Spiroacetal pectenotoxin-2c (PTX2c) was also synthesized from PTX2b. The cytotoxicity assay of the synthetic compounds against HepG2 and Caco2 cancer cells showed a potency of the order: PTX2 > PTX2b > PTX2c.

Pectenotoxin-2 (PTX2; **1**; Figure 1), isolated from the Japanese scallop *Patinopecten yessoensis* and the dinoflagellate *Dinophysis fortii* as a diarrhetic shellfish toxin by Yasumoto et al. in the late 1980s,^[1] is a unique polyether macrolide consisting of a 34-membered (main chain) lactone, a spiroacetal, a bicyclic acetal, three oxolanes, and a six-membered cyclic acetal. PTX2 has natural congeners having differences in the oxidation state at C43 and in the structures of the spiroacetal (C3–C11), as well as the oxolane/hemiacetal (C32–C38) regions.^[1,2] The toxicity of the pectenotoxin congeners (PTXs) is demonstrated by lethality to mice after

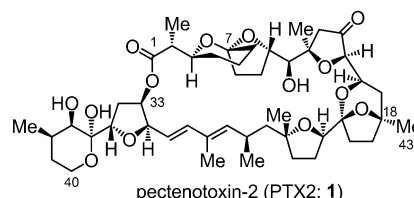
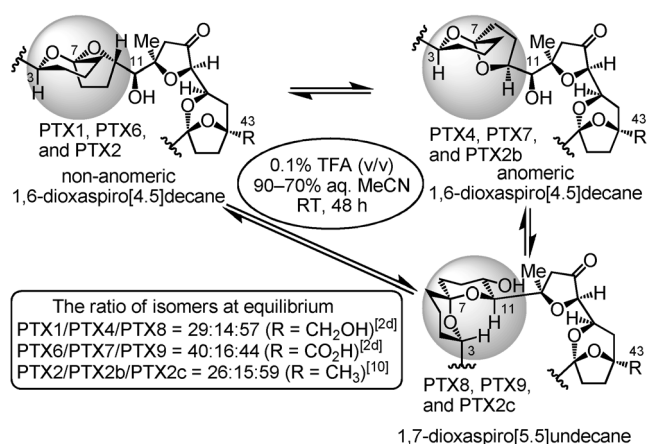


Figure 1. Structure of pectenotoxin-2.

intraperitoneal injection^[1–3] and cytotoxicity to several cancer cell lines,^[4] which is attributable to their actin depolymerization ability.^[5] The toxicity is reported to correlate with their structures. An increase in the oxidation state at C43 and structural deviations from the spiroacetal consisting of six- and five-membered rings with a 7R configuration decreased the toxicity.^[6] Thus, PTX2 displayed the strongest toxicity among the congeners.

It is notable that the spirocyclic acetal of PTX2, similar to that of PTX1 and PTX6 (Scheme 1), takes a form that is not stabilized by an anomeric effect: the oxygen atom at C7



Scheme 1. Reported interconversion between PTX congeners. TFA = trifluoroacetic acid.

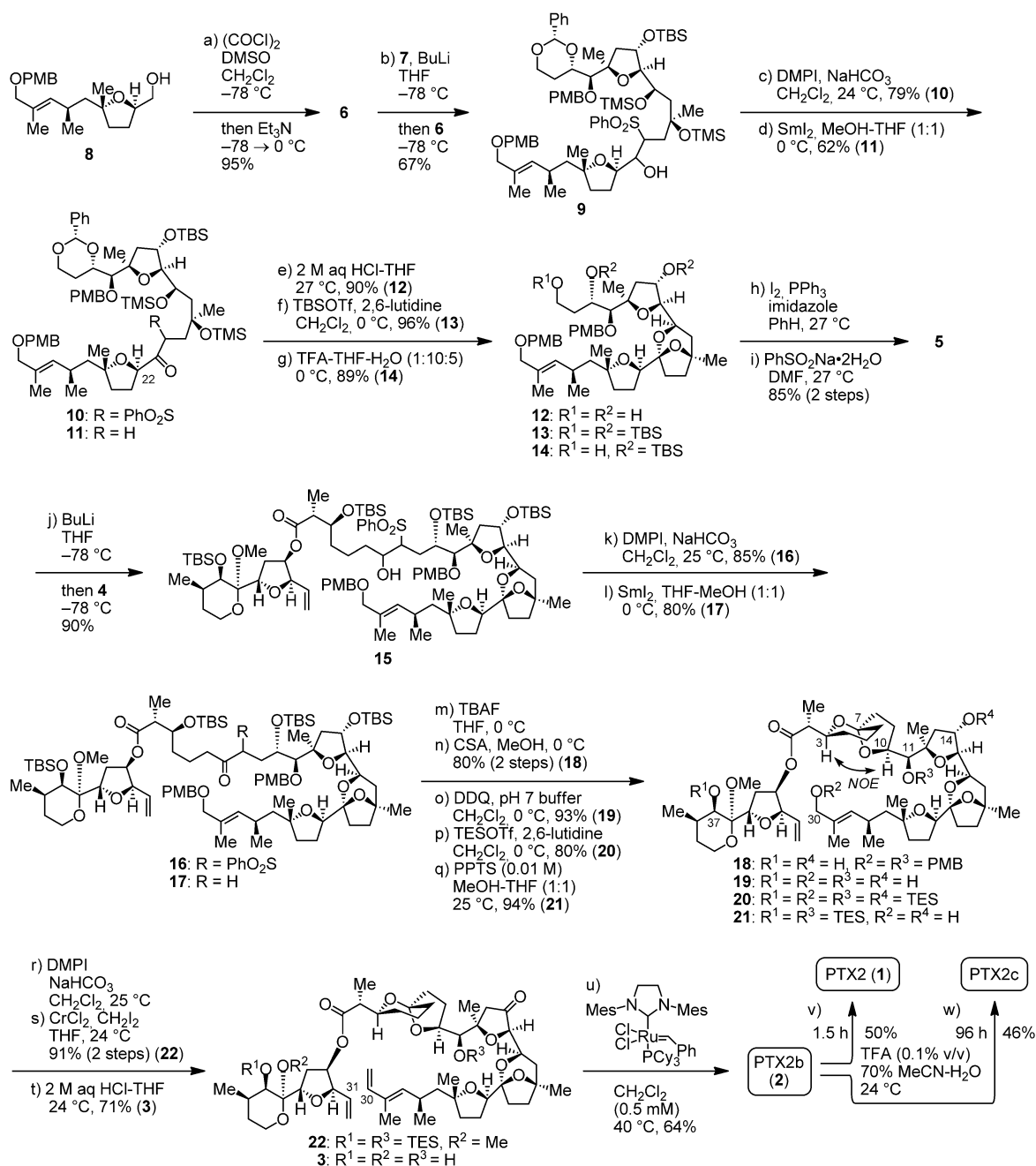
exocyclic to the oxane ring is in the equatorial position. The unique characteristics of the common non-anomeric^[7] spiroacetal moiety of PTX1, PTX2, and PTX6 has attracted the attention of organic chemists and promoted the development of some successful methods for the selective synthesis of non-anomeric spiroacetals, for example, a sequence of stereoselective reductive lithiation and C–C bond formation by Rychnovsky and co-workers,^[8] and stereoselective spiroacetalization under carefully controlled kinetic conditions by Pihko and co-workers.^[9]

[*] Prof. Dr. K. Fujiwara, Y. Suzuki, N. Koseki, Y. Aki, Y. Kikuchi, S. Murata, F. Yamamoto, M. Kawamura, Prof. Dr. A. Murai, Prof. Dr. R. Katoono, Prof. Dr. H. Kawai, Prof. Dr. T. Suzuki
Department of Chemistry, Faculty of Science
Hokkaido University, Sapporo 060-0810 (Japan)
E-mail: ffwkn@sci.hokudai.ac.jp

Prof. Dr. T. Norikura, Prof. Dr. H. Matsue
Department of Nutrition, Faculty of Health Science
Aomori University of Health and Welfare
Mase 58-1, Hamadate, Aomori 030-8505 (Japan)

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Scheme 3. Segment assembly and completion of synthesis. Reagents and conditions: a) (COCl)₂, DMSO, CH₂Cl₂, −78 °C, 10 min; then Et₃N, −78 °C → 0 °C, 20 min, 95%; b) **7**, BuLi, THF, −78 °C, 10 min; then **6**, 10 min, 67%; c) DMPI, NaHCO₃, 24 °C, 2 h, 79%; d) Sml₂, MeOH/THF (1:1), 0 °C, 5 min, 62%; e) 2 M aq. HCl/THF (1:4), 27 °C, 15 h, 90%; f) TBSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C, 1 h, 96%; g) TFA/THF/H₂O (1:10:5), 0 °C, 3 h, 89%; h) I₂, PPh₃, imidazole, benzene, 27 °C, 10 min; i) PhSO₂Na·2H₂O, DMF, 27 °C, 13 h, 85% (2 steps); j) **5**, BuLi, THF, −78 °C, 15 min; then **4**, 20 min, 90%; k) DMPI, NaHCO₃, 25 °C, 2 h, 85%; l) Sml₂, MeOH/THF (1:1), 0 °C, 5 min, 80%; m) TBAF, THF, 0 °C, 16 h; n) CSA, MeOH, 0 °C, 2 h, 80% (2 steps); o) DDQ, CH₂Cl₂/pH 7 buffer (10:1), 0 °C, 1 h, 93%; p) TESOTf, 2,6-lutidine, CH₂Cl₂, 0 °C, 2 h, 80%; q) PPTS (0.01 M), MeOH/THF (1:1), 25 °C, 3 h, 94%; r) DMPI, NaHCO₃, 25 °C, 3 h; s) CrCl₂, CH₂I₂, THF, 24 °C, 15 h, 91% (2 steps); t) 2 M aq. HCl/THF (1:4), 24 °C, 5 h; u) Grubbs' second generation catalyst, CH₂Cl₂ (0.5 mM of **22**), 40 °C, 4 h, 64%; v) TFA (0.1% v/v), 70% MeCN-H₂O, 24 °C, 1.5 h, **1**: 50%, **2**: 18%, PTX2c: 11%; w) TFA (0.1% v/v), 70% MeCN/H₂O, 24 °C, 96 h, **1**: 22%, **2**: 18%, PTX2c: 46%. CSA = 10-camphorsulfonic acid, Cy = cyclohexyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMF = *N,N*-dimethylformamide, DMPI = Dess–Martin periodinane, DMSO = dimethyl sulfoxide, Mes = 2,4,6-trimethylphenyl, NOE = nuclear Overhauser effect, PPTS = pyridinium *p*-toluenesulfonate, TBAF = tetrabutylammonium fluoride, TES = triethylsilyl, Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran.

groups with TBAF, 2) acetal cyclization with CSA] to give anomeric spiroacetal **18** (80% over two steps). The configuration at C7 was confirmed by the presence of an NOE correlation between H3 and H10.^[2d]

Next, conversion of **18** into diol **21**, possessing hydroxy groups at C14 and C30 as well as protected oxygen atoms at C11 and C37, was required prior to the installation of a carbonyl group at C14 and a vinyl group at C30. The

PMB groups of **18** were removed with DDQ (93 %),^[19] and the resulting tetraol **19** was protected as a tetra(TES) ether (**20**; 80 %). The sterically less hindered TES ethers at C14 and C30 were selectively cleaved under weak acidic conditions to produce **21** (94 %).

Dess–Martin oxidation of **21** followed by Takai olefination^[20] selectively afforded ketotriene **22** (91 % over two steps). All protecting groups of **22** were removed upon treatment with 2 M aqueous HCl/THF (1:4) to furnish **3** (71 %) along with a small amount of a [6,6]-spiroacetal (12 %). The RCM of triene **3** with the second-generation Grubbs' catalyst^[21] successfully gave PTX2b (**2**) in 64 % yield without isomerization of the spiroacetal moiety.

The final isomerization of synthetic PTX2b (**2**) was performed using a 0.1 % solution of TFA in 70 % MeCN/H₂O at 24 °C. Prior to the preparative scale reaction, the time course of the isomerization of PTX2b was monitored in detail on an approximate 0.1 mg scale by reverse-phase HPLC with UV detection at $\lambda = 235$ nm (Figure 2).^[22] As predicted,

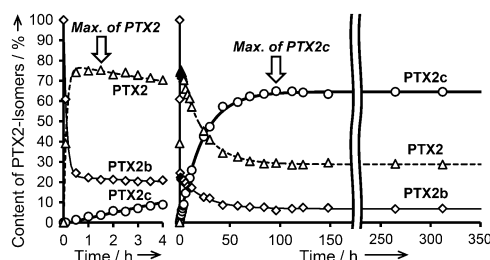


Figure 2. Time course of the isomerization of synthetic PTX2b (**2**) (small-scale experiment).

PTX2b was rapidly isomerized to PTX2 at the initial stage of the isomerization. The content of PTX2 soared from 0 % to a maximum of 75 % after 1.5 hours, while the content of PTX2b decreased steeply from 100 % to 21 % in the same period. Then, PTX2 and PTX2b slowly decreased with a gradual increase of PTX2c until 96 hours when the isomerization reached equilibrium and PTX2c was at a maximum of 65 % (PTX2/PTX2b/PTX2c = 28:7:65). Thus, in preparative scale isomerization reactions, a 1.5 hour reaction produced **1** as the major component in 50 % upon isolation, and a 96 hour reaction led to the isolation of PTX2c (46 %).^[23]

The synthetic PTX2 (**1**) displayed physical and spectral properties including ¹H and ¹³C NMR, IR, UV, and MS data, as well as optical rotation $\{[\alpha]_D^{23} = +15.5$ ($c = 0.050$, MeOH); Ref. [1a]: $[\alpha]_D^{20} = +16.2$ ($c = 0.105$, MeOH) $\}$, identical to the natural product.^[1a,2c,f] The structures of synthetic PTX2b and PTX2c were determined by an independent NMR analysis (see the Supporting Information), and the order of elution of the synthetic congeners from HPLC agreed with that of the naturally derived congeners,^[10] thereby confirming Suzuki's proposed structures of PTX2b and PTX2c.

The cytotoxicity against human hepatocellular carcinoma cells (HepG2) and human colonic carcinoma cells (Caco2), which was previously examined with natural PTX2 by Sérandour and co-workers,^[24] was then investigated with the synthetic compounds. Non-anomeric spiroacetal PTX2

showed the highest IC₅₀ value against HepG2/Caco2 growth (3.6/2.6 nmol L⁻¹), while the anomeric spiroacetal PTX2b and [6,6]-spiroacetal PTX2c displayed IC₅₀ values of 1.5×10^3 / 0.56×10^3 and 3.8×10^3 / 3.6×10^3 nmol L⁻¹, respectively. Thus, the difference in cytotoxicity between spiroacetalic isomers of PTX2 was clarified for the first time, though a similar structure–activity relationship was reported in the mouse lethality of PTX1, PTX4, and PTX8 by Yasumoto.^[2d]

In conclusion, the total synthesis of PTX2 (**1**) was achieved through a simple process including sulfone-mediated assembly of bicyclic and anomeric spirocyclic acetals from segments **6**, **7**, and **4**, macrocyclization through RCM to form PTX2b (**2**), and acid-catalyzed isomerization of anomeric spirocyclic acetal **2** to non-anomeric spirocyclic acetal **1** by the early termination of a dynamic transition process to equilibrium. [6,6]-Spiroacetal PTX2c was also synthesized from **2**. The cytotoxicity assay of the synthetic compounds against HepG2 and Caco2 cancer cells showed a potency order of PTX2 >> PTX2b > PTX2c.

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- [22] For the reaction conditions of the isomerization of **2**, see the Supporting Information.
- [23] The isomerization of pure PTX2c was also examined under the same reaction conditions. As a result, PTX2c slowly decreased with gradually increasing PTX2 and PTX2c until after 92 h when the isomerization mixture was at equilibrium (PTX2/PTX2b/PTX2c = 24:7:69). See the Supporting Information.
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