J|A|C|S
ARTICLES
Published on Web 06/28/2003

# First Asymmetric Total Synthesis of Tetrodotoxin

Norio Ohyabu, Toshio Nishikawa, and Minoru Isobe*

*Contribution from the Laboratory of Organic Chemistry, School of Bioagricultural Sciences, Nagoya University, Furocho, Chikusa, Nagoya 464-8601, Japan*

Received January 23, 2003; E-mail: isobem@agr.nagoya-u.ac.jp

***Abstract:*** Tetrodotoxin, a toxic principle of puffer fish poisoning, is one of the most famous marine natural products because of the complex structure having many functional groups and its potent biological activity leading to death. Since the structure elucidation in 1964, this toxin has been recognized as a formidable target molecule for total synthesis. We have recently achieved the first asymmetric total synthesis from 2-acetoxy-tri-*O*-acetyl-D-glucal as a chiral starting material. The highly hydroxylated cyclohexane ring was constructed by Claisen rearrangement and regioselective hydroxylations of an acetone moiety and an intramolecular directed aldol condensation of the precursor having methyl ketone with dihydroxyacetone, which was synthesized through Sonogashira coupling. Installation of nitrogen functionality was unsuccessful through an attempted Overman rearrangement. We, therefore, employed a new intramolecular conjugate addition strategy between the carbamate and unsaturated ester groups. The α-hydroxyl lactone moiety was synthesized through an intramolecular epoxide opening by the *Z*-enolate of aldehyde, which was followed by oxidation−reduction of the resulting cyclic vinyl ether. The lactone was then converted to a protected ortho ester, and then gunanidinylation was followed by cleavage of the 1,2-glycol to give the fully protected tetrodotoxin. Selection of the protective groups has finally led us to accomplish the total synthesis of tetrodotoxin in an enantiomerically pure form. All the stereogenic centers were controlled with high selectivity, and the hydroxyl groups were differently protected to discriminate for the future analogue synthesis of a bioorganic program. The synthetic tetrodotoxin was purifed by ion exchange chromatography and characterized to be identifical with the natural compound.

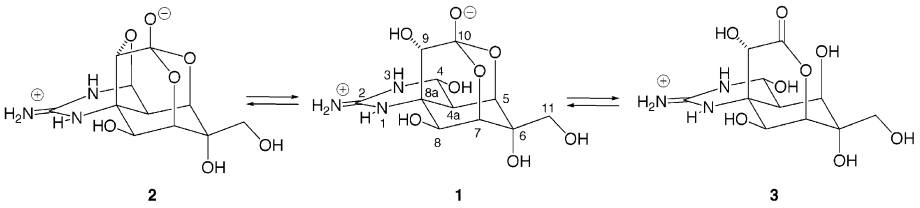
## Introduction

Puffer fish poisoning has been a very famous yet serious problem, especially in Japan, where the puffer fish has been long time recognized as one of the most delicious seafoods.[1] The toxic principle was first isolated from the ovaries of the puffer fish (*Spheroides rubripes*) in 1909 and named as tetrodotoxin (TTX) after the puffer fish family "Tetraodontidae."[2] Despite the small molecule, however, the structure elucidation had been extremely difficult at the time, because of the unusual chemical properties and the unprecedented structure. After extensive efforts, three groups including Hirata-Goto,[3] Tsuda,[4] and Woodward[5] independently arrived at the same structure **1** (Figure 1) for tetrodotoxin.[6] Absolute stereochemistry of tetrodotoxin **1** was determined unambiguously by X-ray crystallographic analysis of its derivative.[7] The structural features are an unprecedented dioxa-adamantane skeleton functionalized by hydroxyl groups, an ortho ester showing acidity ($pK_a = 8.7$), and cyclic guanidine with hemiaminal. Tetrodotoxin exists as an equilibrium mixture among the ortho ester (**1**), anhydride (**2**), and lactone (**3**) forms, as shown in Figure 1.

Since the toxicity of tetrodotoxin was revealed in the 1960s to be attributed to a specific blockage of sodium ion influx through sodium channel proteins, tetrodotoxin has been widely employed in pharmacological studies.[8] And further extensive studies culminated in the identification and isolation of the ion-channel protein and determination of its amino acid sequence.[9] Concerning the total synthesis of this fascinating and challenging natural product, Kishi−Goto and their co-workers achieved the racemic total synthesis in 1972.[10] Since then, there have been extensive synthetic efforts devoted to this small natural product,[11] yet the Kishi−Goto total synthesis had still stood as the only synthesis of tetrodotoxin **1**. The bioorganic studies were reported through a difficult derivatization from natural tetro-

(1) Review: Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel. Kao, C. Y.; Lovinson, S. Eds. *Ann. N. Y. Acad. Sci.* **1986**, *479*.
(2) (a) Tahara, Y. *J. Pharm. Soc. Jpn. (in Japanese)* **1909**, *29*, 587−625. (b) Tahara, Y. *Biochem. Z.* **1911**, *30*, 255−275.
(3) Goto, T.; Kishi, Y.; Takahashi, S.; Hirata, Y. *Tetrahedron* **1965**, *21*, 2059−2088.
(4) Tsuda, K.; Ikuma, S.; Kawamura, M.; Tachikawa, R.; Sakai, K.; Tamura, C.; Amakasu, O. *Chem. Pharm. Bull.* **1964**, *12*, 1357−1374.
(5) Woodward, R. B. *Pure. Appl. Chem.* **1964**, *9*, 49−74.
(6) The structure was finally confirmed by X-ray analysis of tetrodotoxin hydrobromide. See: Furusaki, A.; Tomie, Y.; Nitta, I. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3332−3341.
(7) Furusaki, A.; Tomie, Y.; Nitta, I. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3325−3331.
(8) (a) Narahashi, T.; Moore, J. W.; Scott, W. R. *J. Gen. Physiol.* **1964**, *47*, 965−974. (b) Hucho, F. *Angew. Chem, Int. Ed. Engl.* **1995**, *34*, 39−50.
(9) Review: Numa, S.; Noda, M. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 338−355.
(10) (a) Kishi, Y.; Aratani, M.; Fukuyama, T.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura, S.; Kakoi, H. *J. Am. Chem. Soc.* **1972**, *94*, 9217−9219. (b) Kishi, Y.; Fukuyama, T.; Aratani, M.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura, S.; Kakoi, H. *J. Am. Chem. Soc.* **1972**, *94*, 9219−9221.
(11) For leading references from other laboratories, see: (a) Itoh, T.; Watanabe, M.; Fukuyama, T. *Synlett* **2002**, 1323−1325. (b) Noya, B.; Paredes, M. D.; Ozores, L.; Alonso, R. *J. Org. Chem.* **2000**, *65*, 5960−5968. (c) Fraser-Reid, B.; Burgey, C. S.; Vollerthun, R. *Pure Appl. Chem.* **1998**, *61*, 285−288. (d) Sato, K.; Kajihara, Y.; Nakamura, Y.; Yoshimura, J. *Chem. Lett.* **1991**, 1559−1562. (e) Nachman, R. J.; Hönel, M.; Williams, T. M.; Halaska,

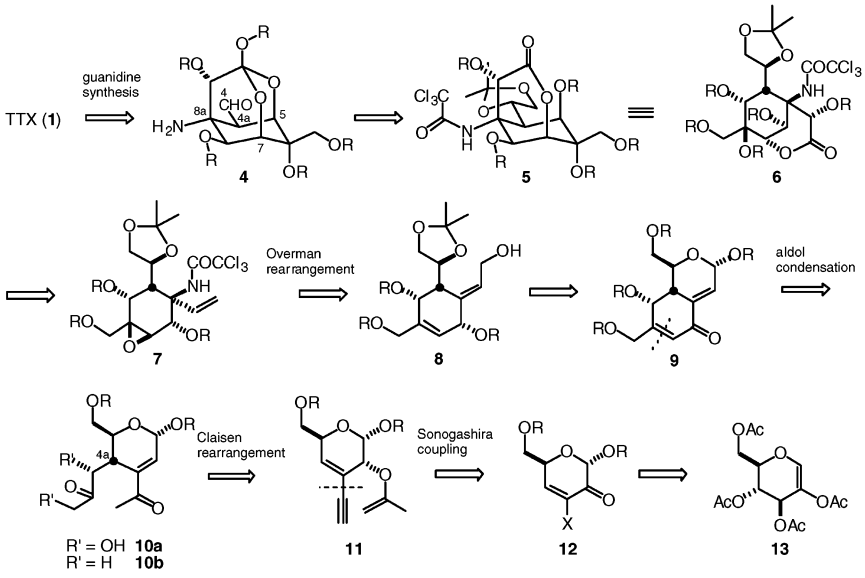

10.1021/ja0342998 CCC: $25.00 



**2** **1** **3**

***Figure 1.*** Equilibrium of tetrodotoxin.

***Scheme 1.*** Retrosynthesis and Synthetic Plan




dotoxin.[12] Therefore, we need analogous compounds through chemical synthesis.

In our project directed toward the total syntheses of tetrodotoxin and its natural and unnatural analogues, we have recently completed the syntheses of 5,11-dideoxytetrodotoxin,[13] 11-deoxytetrodotoxin,[14] and 8,11-dideoxytetrodotoxin[15] in enantiomerically pure form on the basis of a Diels−Alder strategy from levoglucosenone. Among them, 11-deoxytetrodotoxin was the first total synthesis of a naturally occurring tetrodotoxin analogue. The full details of the total synthesis of tetrodotoxin (**1**) are described here on a different basic strategy aiming at a higher oxidation stage in earlier stages of the synthesis.

## Retrosynthesis and Synthetic Plan

For the asymmetric synthesis toward **1**, **2**, and **3**, we had aimed at retrosynthesis ultimately into a readily available sugar derivative through the chemistry which we have learned during the course of the TTX-analogue syntheses. The cyclic guanidine moiety with hemiaminal has been prepared from a guanidine group at the C-8a position and an aldehyde at the C-4 position.[16] An intermediate **4** bearing ortho ester and amino aldehyde was envisaged as the synthetic equivalent of tetrodotoxin (Scheme 1).[17] A similar ortho ester intermediate was, in fact, crucial for successful synthesis of 8,11-dideoxytetrodotoxin.[15] Employment of the ortho ester instead of $\delta$-hydroxyl lactone seemed to be more advantageous not only for the protecting the carboxylic acid with two hydroxyl groups at the C-5 and C-7 positions but also for preventing the C-5 hydroxyl function from $\beta$-elimination.[18] The ortho ester form would also inhibit epimerization at the C-9 position.[19] The amino group, a pivotal function for guanidine installation, would be protected as trichloroacetamide, while the aldehyde could be prepared from 1,2-glycol protected as acetonide. These analyses led us to find the lactone structures (**5**, **6** as three- and two-dimensional expressions) as an important intermediate. According to the successful syntheses of deoxytetrodotoxins in our laboratory, the lactone **6** was then simplified into vinyl epoxide **7**. It was anticipated that nitrogen functionality would be introduced through Overman rearrangement[20] from *exo*-allylic alcohol **8**, in which the stereochemical course should be controlled by allylic strain between acetonide and allylic trichloroacetimidate.[21] Consequently, one of the main issues in the current plan was stereocontrolled synthesis of the

R. C.; Mosher, H. S. *J. Org. Chem.* **1986**, *51*, 4802−4806. (f) Keana, J. F. W.; Bland, J. S.; Boyle, P. J.; Erion, M.; Hartling, R.; Husman, J. R.; Roman, R. B. *J. Org. Chem.* **1983**, *48*, 3621−3631. (g) Speslacis, J. Ph.D. Thesis, Harvard University, 1975.

(12) (a) Balerna, M.; Lombet, A.; Chicheportiche, R.; Romey, G.; Lazdunski, M. *Biochim. Biophys. Acta.* **1981**, *644*, 219−225. (b) Bontemps, J.; Cantineau, R.; Grandfils, C.; Leprince, P.; Dandrifosse, G.; Schoffeniels, E. *Anal. Biochem.* **1984**, *139*, 149−157. (c) Wu, B. Q.; Yang, L.; Kao, C. Y.; Levinson, S. R.; Yotsu-Yamashita, M.; Yasumoto, T. *Toxicon* **1996**, *34*, 407−416. (d) Nakayama, H.; Hatanaka, Y.; Yoshida, E.; Oka, K.; Takanohashi, M.; Amano, Y.; Kanaoka, Y. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 900−907.

(13) (a) Nishikawa, T.; Asai, M.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 3081−3084. (b) Asai, M.; Nishikawa, T.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Tetrahedron* **2001**, *57*, 4543−4558.

(14) Nishikawa, T.; Asai, M.; Isobe, M. *J. Am. Chem. Soc.* **2002**, *124*, 7847−7852.

(15) Nishikawa, T.; Urabe, D.; Yoshida, K.; Iwabuchi, T.; Asai, M.; Isobe, M. *Org. Lett.* **2002**, *4*, 2679−2682.

(16) The numbering used in this paper corresponds to that of tetrodotoxin.

(17) The compound **5** is an ortho ester form of a tetrodamine intermediate named by Kishi. See ref 10.

(18) This problem was mentioned in Kishi's total synthesis; see ref 10.

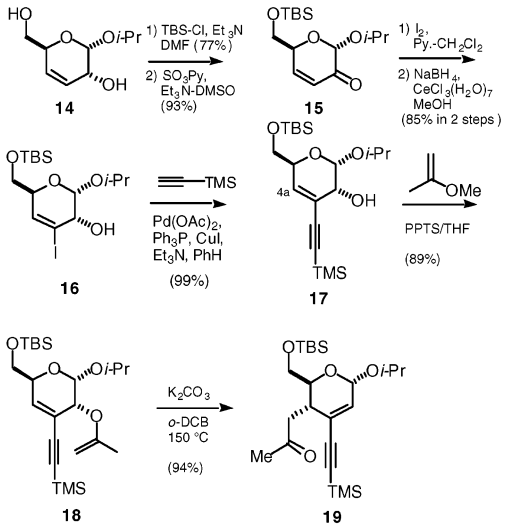
(19) Since the problem was encountered in the synthesis of (−)-5,11-dideoxytetrodotoxin, we devised intramolecular acetal between the hydroxyl group at the C-9 position and aldehyde at the C-4 position to protect the labile C-9 position. See ref 13.

oxygenated precursor **8** for Overman rearrangement. In our previous retrosynthesis toward the deoxy tetrodotoxin analogues, a Diels−Alder cycloaddition strategy was employed for the cyclohexene ring construction.[22] An alternative strategy was based on electrocyclization for the cyclohexene moiety.[23] Besides these two basic strategies, we added the third strategy for the synthesis of the oxygenated cyclohexane skeleton as **8** via a different route. Exoolefin **8** could be prepared from dienone **9** through a directed aldol cyclization of **10** and Claisen rearrangement of **11** as conceptual key steps. The enone was envisaged to derive from an intramolecular aldol condensation of **10a**; methyl ketone could be prepared by hydration of acetylene, while the dihydroxyacetone moiety might need regio- and stereoselective hydroxylation to the acetone moiety in **10b**. The acetone at the C-4a position would be obtainable via Claisen rearrangement of allyl isopropenyl ether **11**. The ethynyl group would be introduced via Sonogashira coupling of **12** (X = halogen) with an acetylene. We have accessed the readily available 2-acetoxy-tri-*O*-acetyl-D-glucal **13** as the chiral starting material.

### Synthesis of the Cyclohexane Skeleton

Synthesis started with allylic alcohol **14**, readily prepared in two steps from 2-acetoxy-tri-*O*-acetyl-D-glucal **13** (Scheme 2).[24] The primary alcohol of **14** was protected with *tert*-butyldimethylsilyl chloride (TBS-Cl) in the presence of triethylamine, while the secondary alcohol was oxidized with $SO_3$•Py, DMSO, and $Et_3N$ (Parikh−Doering oxidation)[25] to give enone **15**. Iodination of **15** was carried out with iodine in pyridine and $CH_2Cl_2$[26] to give α-iodoenone, which was subsequently reduced under Luche's conditions[27] to **16** as a single product. Sonogashira coupling[28] of iodide **16** with (trimethylsilyl)acetylene gave eneyne **17** in quantitative yield. Acetone moiety was installed to the C-4a position of **17** via Claisen rearrangement of allyl-2-propenyl ether **18**. Thus, **17** was treated with 2-methoxypropene in the presence of pyridinium *p*-toluenesulfonate (PPTS) to give a mixture of unstable propenyl ether **18**, methyl acetal, and the starting alcohol **17**, which were separated on silica gel (neutral) column chromatography to give **18** in 56% yield.[29] The recovered materials could be converted to **18** under the same conditions. After three cycles, an overall yield of 89% was obtained for **18**. The Claisen rearrangement of **18** proceeded upon heating at 150 °C in *o*-dichlorobenzene (*o*-DCB) in the presence of potassium carbonate[30] to afford **19** as the only isolable product in high yield.

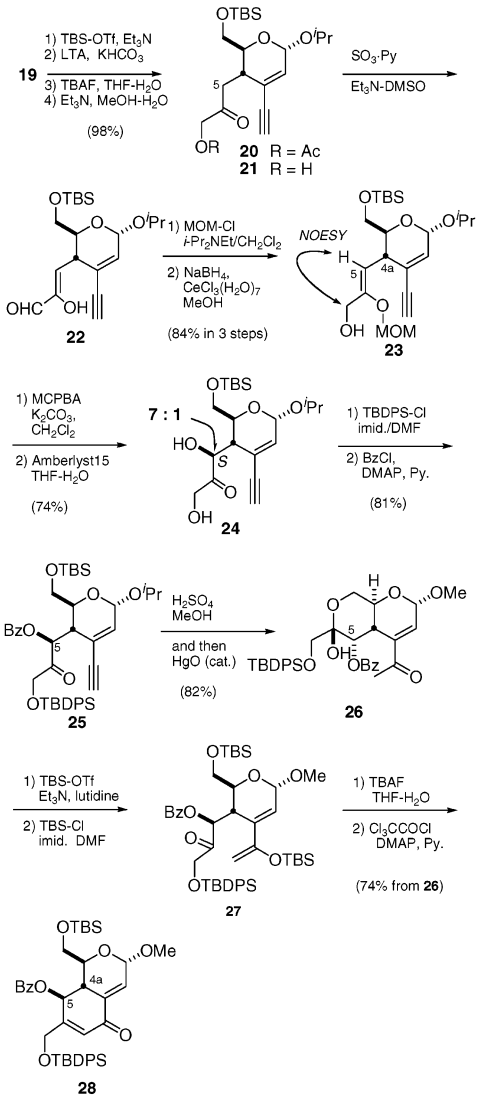
***Scheme 2.*** Sonogashira Coupling and Claisen Rearrangement Strategy




Hydroxylations of both neighboring sides of the acetone moiety were carried out stepwise by regioselective enolization followed by oxygenation (Scheme 3). The ketone **19** was first treated with TBSOTf and triethylamine to give exclusively the corresponding vinyl silyl ether at the terminal position, which was then oxidized with lead tetraacetate (LTA).[31] The resulting crude products, presumably an acetyl silyl acetal, were exposed to *n*-$Bu_4NF$ (TBAF) to give acetoxyacetone **20**, which was deacetylated to afford hydroxyacetone **21** in excellent overall yield. Hydroxylation of the internal position was difficult due to little enolization toward the C-5 position, so **21** was once converted to α-aldehyde ketone, and then it was tried for the enolization due to dipole−dipole interaction. Thus, upon Parikh−Doering oxidation of **21**, the resulting α-aldehyde ketone spontaneously enolized to **22** as an unstable product. After protection of the enol as methoxymethyl (MOM) ether, the aldehyde was reduced under the Luche's reductions to give **23**. Geometry of the enol ether was established to be *Z* from NOESY correlation as shown in structure **23**. Epoxidation of **23** with *m*-chloroperbenzoic acid (MCPBA) in the presence of potassium carbonate[32] gave unstable products, which were directly hydrolyzed with Amberlite 15 ion-exchange resin ($H^+$ form) to give dihydroxylacetone **24** as a 7:1 mixture of the diastereomers; thus, the major product was separated and isolated in 74% yield along with the minor isomer in about 10% yield. As it turned out later, the configuration of C-5 was revealed to be *S*. The stable conformation of enol ether **23** is shown in Figure 2, which was assigned from two large coupling constants (10.5 Hz both) between H-4 and H-4a and H-4a and H-5 in the $^1H$ NMR spectra. This diastereomeric selectivity (ca. 7:1) might be rationalized by the cooperative effect[33] of hydrogen bonds of MCPBA with the allylic alcohol and oxygen

(20) For reviews of the Overman rearrangement, see: (a) Overman, L. E. *Acc. Chem. Res.* **1980**, *13*, 218−224. (b) Ritter, K. In *Houben-Weyl. Stereoselective Synthesis*. E 21; Helmchen, G., Hoffmann, R. W., Mulzer, J., Schaumann, E., Eds.; Thieme: Stuttgart, Germany, 1996; Vol. 9, pp 5677−5699.
(21) Isobe, M.; Fukuda, Y.; Nishikawa, T.; Chabert, P.; Kawai, T.; Goto, T. *Tetrahedron Lett.* **1990**, *31*, 3327−3330.
(22) Isobe, M.; Nishikawa, T.; Pikul, S.; Goto, T. *Tetrahedron Lett.* **1987**, *28*, 6485−6488. (b) Isobe, M.; Yamamoto, N.; Nishikawa, T. In *Levoglucosenone and Levoglucosans, Chemistry and Applications*. Witczak, Z. J. Ed.; ATL Press: Mount Prospect, IL, 1994; pp 99−118.
(23) Bamba, M.; Nishikawa, T.; Isobe, M. *Tetrahedron* **1998**, *54*, 6639−6650.
(24) Ichikawa, Y.; Isobe, M.; Bai, D.-L.; Goto, T. *Tetrahedron* **1987**, *43*, 4737−4776.
(25) Parikh, J. R.; Doering, W. von E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.
(26) Johnson, C. R.; Adams, J. P.; Braun, M. P.; Senanayke, C. B. W.; Wovkulich, P. M.; Uskokovic, M. R. *Tetrahedron Lett.* **1992**, *33*, 917−918.
(27) Germal, A. L.; Luche, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 5454−5459.
(28) Sonogashira, K.; Tohda, Y.; Hagiwara, N. *Tetrahedron Lett.* **1975**, *16*, 4467−4470.
(29) Since the product **18** was sensitive to acid as silica gel, neutral silica gel with eluent containing a small amount of triethylamine was employed for this chromatography. For details, see experimental procedure in the Supporting Information.
(30) In the absence of $K_2CO_2$, some degree of hydrolysis to **17** was observed.
(31) (a) Rubottom, G. M.; Gruber, J. M.; Kincaid, K. *Synth. Commun.* **1976**, *6*, 59−62. (b) Rubottom, G. M.; Gruber, J. M. *J. Org. Chem.* **1976**, *41*, 1673−1674.
(32) In the presence of $Na_2HPO_4$ instead of $K_2CO_3$, the resulting epoxides were partially incorporated with the residual *m*-chlorobenzoic acid to give a mixture of **24** and the byproducts, which upon hydrolysis ($K_2CO_3$, MeOH) were converted to **24** in ca. 30% yield from **23**.

***Scheme 3.*** Synthesis of the Cyclohexenone




of the siloxy group of **23** as depicted in Figure 2b rather than steric hindrance of the TBS group. Although the configuration was opposite for tetrodotoxin, the major product **24** was employed for further studies with success.

Selective protection of the primary alcohol of **24** with a *tert*-butyldiphenylsilyl (TBDPS) group and subsequent benzoylation of the secondary alcohol gave **25**. Acetylene **25** was successively treated with sulfuric acid and a catalytic amount of mercuric oxide to afford methyl ketone **26**. Thus, with treatment with sulfuric acid in methanol, the TBS group was immediately removed to form intramolecular acetal, and then *iso*-propyl acetal was replaced with methanol. When mercuric oxide was added to the resulting mixture, acetylene underwent hydration to methyl ketone **26** in 82% overall yield. With this bicyclic compound, the configuration of the C-5 position was established to be *S* by a small coupling constant of 2 Hz between protons at C-5 and C-4a in the $^1$H NMR spectra, indicating axial arrangement of the C-5 benzoyloxy group (Figure 3a).[34] The methyl ketone of **26** was then treated with TBSOTf in the presence of $Et_3N$ and 2,6-lutidine to give alkenyl silyl ether,

(33) (a) Johnson, M. R.; Kishi, Y. *Tetrahedron Lett.* **1979**, 4347−4350. (b) Isobe, M.; Kitamura, M.; Mio, S.; Goto, T. *Tetrahedron Lett.* **1982**, *23*, 221−224.

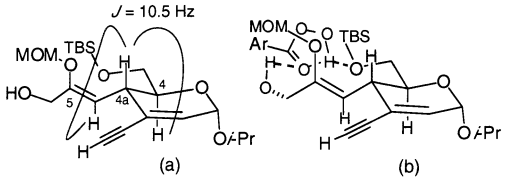



***Figure 2.*** (a) Conformation of **23** and (b) a possible interaction with MCPBA.

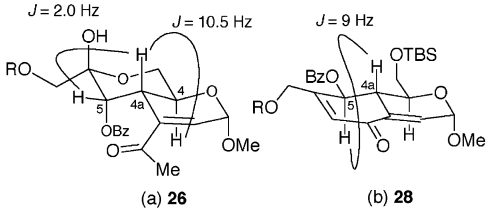
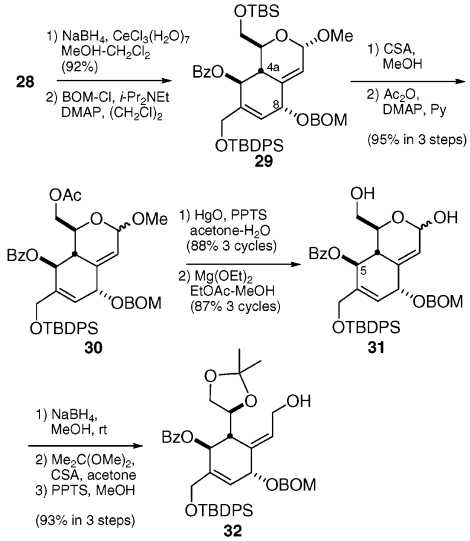


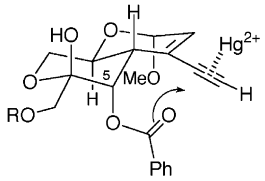

***Figure 3.*** Proof of the stereochemistry at the C5 position.

***Scheme 4.*** Exoolefin Synthesis




which was further silylated with TBS-Cl in the presence of imidazole to afford **27**,[35] a precursor for directed aldol condensation. Intramolecular aldol reaction was carried out by *n*-$Bu_4$NF (TBAF),[36] and subsequent dehydration with trichloroacetyl chloride afforded enone **28** in good overall yield. The large coupling constant, 9 Hz between H-5 and H-4a, confirmed the configuration of the C-5 position as well as the trans diaxial relationship of these two protons (Figure 3b).

(34) Interestingly, configuration of the C-5 position affected hydration of acetylene; the reaction of the *R* isomer under the identical conditions did not give the corresponding product. The hydration of the acetylene **25** without the hydroxyl group at the C-5 position necessitated harsh conditions (2 equiv of HgO in 1.26 M aq $H_2SO_4$:acetone = 1:1, under reflux for 30 min) to give the corresponding ketone in 78% yield. These results imply the benzoate ester group of *S*-isomer might participate the acetylene moiety in the hydration as shown below.




(35) Two step silylation was necessary for high yield of the product **27**. Under the first conditions, silylation of the primary alcohol was not effective to give a much lower yield of **27**.

(36) Because of intramolecular reaction, water did not interfere with the fluoride induced aldol reaction.

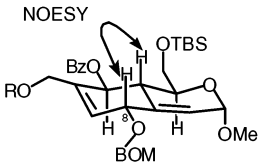


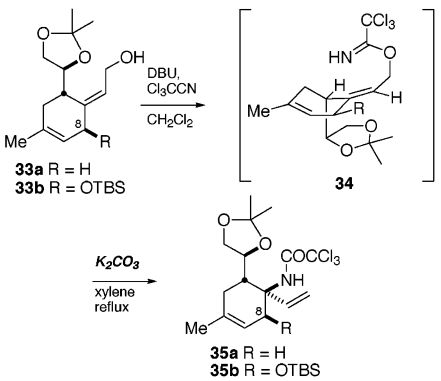

***Figure 4.*** Proof of the configuration of the C-8 position.

The enone of **28** was reduced under Luche's conditions to give α-alcohol as a single product (Scheme 4). The configuration of the resulting alcohol was determined by observing the NOESY correlation between the protons of the C-4a and C-8 positions of the corresponding BOM (benzyloxymethyl) ether **29** (Figure 4). Further conversion to *exo*-allylic alcohol **32**, a precursor for the Overman rearrangement, proved to be difficult because of facile intramolecular acetal (1,6-anhydro ring in sugar numbering) formation and instability of the hydroxyl group at the double allylic C-8 position. In this specific case, the resulting anhydro byproduct could not be converted to **32** due to the presence of a labile alkoxy group at the C-8 position. Thus, the BOM group was carefully chosen as the protective group for the C-8 hydroxyl group because of the low leaving ability, high compatibility with the further transformation, and the mild deprotection conditons. The TBS ether of **29** was converted to the corresponding acetate **30** through desilylation with *dl*-camphorsulfonic acid (CSA) in methanol followed by acetylation. Protection of the primary alcohol was necessary to prevent 1,6-anhydro ring formation under the next reaction. Hydrolysis of the acetal **30** was best carried out by mercuric oxide,[37] and further treatment with PPTS in aqueous acetone and subsequent mild deacetylation with $Mg(OEt)_2$[38] afforded **31**. The hemiacetal of **31** was easily reduced with sodium borohydride, and the resulting triol was protected as acetonide to furnish **32** in good overall yield.[39] This is now set for the Overman rearrangement.

## Introduction of Nitrogen Functionality

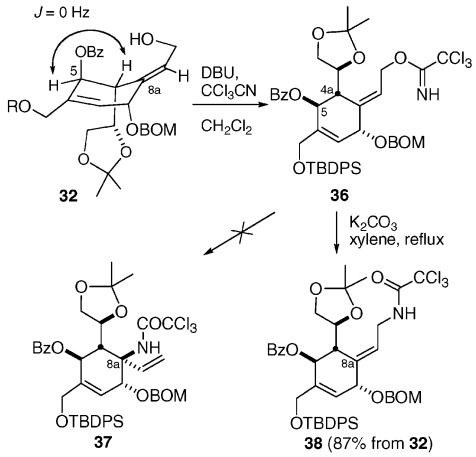
In the synthesis of the common intermediate **35a** aiming at a variety of analogues of tetrodotoxin (Scheme 5), we developed an improved condition ($K_2CO_3$ in xylene, reflux) for the Overman rearrangement of simple *exo*-allylic alcohol **33a** to give **35a** in high yield with reproducibility.[40] The improved condition enabled us to effect the Overman rearrangement of hydroxylated substrate **33b** to afford **35b**.[41] These successful results encouraged us to employ the more hydroxylated substrate **32** as a precursor for the Overman rearrangement.

***Scheme 5.*** Previous Examples of Overman Rearrangement




Thus, imidate **36** was prepared by treatment of **32** with 1,8-diazabicylco[5.4.0]undec-7-ene (DBU) and trichloroacetonitrile (Scheme 6). Considering the allylic strain observed in **34**, we expected the conformation of **32** to be suitable for the rearrangement, as shown in Scheme 6.[42] To our surprise, however, the attempted Overman rearrangement of **36** even under the improved conditions (at xylene reflux in the presence of $K_2CO_3$) did not give the desired product **37** but gave 1,3-shift product **38** as an only isolable product.[43] In this case, the benzoyloxy substituent occupied the axial position of the C-5,[44] which might be steric hindrance against incoming nitrogen of imidate **36**. However, the corresponding epimer at the C-5 position[45] also gave **38** under the same conditions. These unsuccessful results enforced us to explore an alternative route for installation of nitrogen functionality to the highly congested C-8a position.

***Scheme 6.*** Problem of Overman Rearrangement

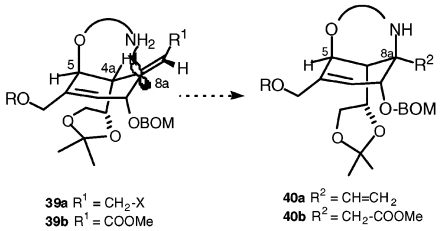



To overcome this critical problem, we probed alternative intramolecular processes for this purpose. As the hydroxyl group of the C-5 position in **32** was located at the axial position, a possible intramolecular $S_N2'$ reaction to an allyl function (**39a**) or conjugate addition to an unsaturated ester (**39b**) appeared to be the candidates (Scheme 7) for the nitrogen nucleophile tethering to the hydroxyl group at the C-5 position.

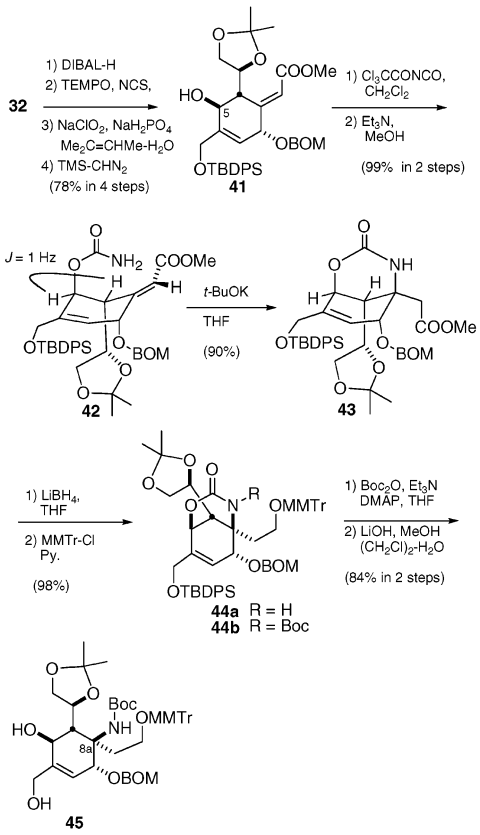
After some exploratory experiments, we found that conjugate addition of a carbamate was feasible as a nitrogen nucleophile

(37) This hydrolysis was accelerated with mercuric oxide. In the absence of HgO, harsh conditions (e.g., CSA (100 mg/mL) in aqueous acetone, room temp, 1 day) were necessary to hydrolyze the methyl acetal to give a mixture of the desired product and the 1,6-anhydro product in 53% and 30% yield, respectively.
(38) Xu, Y.-C.; Bizuneh, A.; Walker, C. *Tetrahedron Lett.* **1996**, *37*, 455−458.
(39) In sharp contrast, harsh conditions with $LiAlH_4$ in refluxing $Et_2O$ were required for the reduction of the simpler hemiacetal for the synthesis of **33a** (Scheme 5), while $NaBH_4$ at rt did not reduce the same hemiacetal.
(40) Nishikawa, T.; Asai, M.; Ohyabu, N.; Yamamoto, N.; Fukuda, Y.; Isobe, M. *Tetrahedron* **2001**, *57*, 3875−3883.
(41) Nishikawa, T.; Asai, M.; Ohyabu, N.; Isobe, M. *J. Org. Chem.* **1998**, *63*, 188−192.
(42) This analysis was supported by a coupling constant (0 Hz) between H5 and H4a of $^1H$ NMR spectra of **32**.
(43) Similar types of the undesired products in the Overman rearrangement of highly oxygenated allylic imidate were reported; see: (a) Gonda, J.; Bednarikova, M. *Tetrahedron Lett.* **1997**, *38*, 5569−5572. (b) Eguchi, T.; Kakinuma, K. *Yuki Gosei Kagaku Kyokaishi* **1997**, *55*, 814−823.
(44) The axial alignment was supported by a very small coupling constant between H5 and H-4a.
(45) The epimer was prepared from the epimer of **32**, which was synthesized from **29** in 11 steps including reductive removal of benzoate with DIBAL-H followed by inversion of the configuration through oxidation−reduction and the similar transformation as in the case of **32**.

***Scheme 7.*** Intramolecular Conjugate Addition for Nitrogen Introduction




***Scheme 8.*** Installation of the Nitrogen Functionality



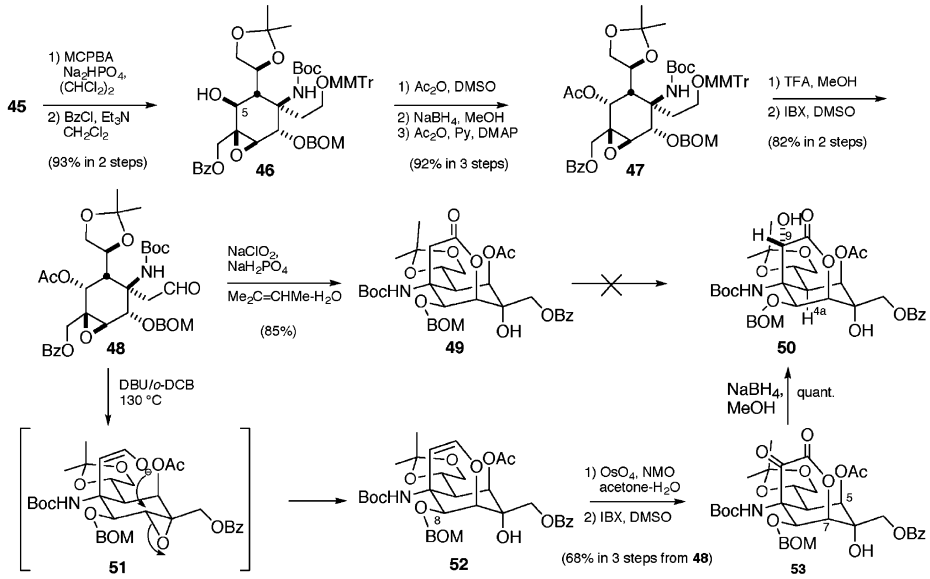

to unsaturated ester (Scheme 8).[46] Thus, the benzoate of **32** was removed with diisobutylaluminum hydride (DIBAL-H), while the primary alcohol was selectively oxidized to carboxylic acid in two steps including 2,2,6,6,-tetramethyl-1-piperdinyloxy, free radical (TEMPO) oxidation[47] followed by sodium chlorite oxidation.[48] Methylation of the resulting carboxylic acid with (trimethylsilyl)diazomethane ($TMSCHN_2$)[49] gave methyl ester **41** in good overall yield from **32**. The remaining hydroxyl group at the C-5 position was then transformed into carbamate **42** in two steps.[50] Intramolecular conjugate addition of **42** was best carried out with potassium *t*-butoxide in THF at −78 to −15 °C to give cyclic carbamate **43** in 90% yield. High yield of the reaction at low temperatures might be due to the dominant conformation as shown in **42**, which was suitable for the intramolecular conjugate addition.[51] Alkaline cleavage of cyclic carbamate **43** through the *N*-Boc intermediate gave rise to the retro Michael reaction with concomitant aromatization of the cyclohexane ring. Consequently, the ester group of **43** was first reduced with $LiBH_4$, and the resulting alcohol was protected as *p*-methoxyphenyldiphenylmethyl (MMTr) ether **44a**. The cyclic carbamate of **44a** was then cleaved through *N*-Boc intermediate **44b** followed by hydrolysis with LiOH[52] to afford **45**. Under the alkaline conditions, the TBDPS group was also removed. Here, the Boc-protected amino group, an important function for guanidinylation, was introduced at the C-8a position.

## Stereoselective Synthesis of the Lactone Ring

Allylic alcohol **45** was epoxidized with MCPBA and then the primary alcohol was protected as benzoate **46** (Scheme 9). The configuration of the C-5 position was inverted by a two-step sequence involving oxidation with Albright-Goldman procedure (acetic anhydride and DMSO)[53] and reduction with $NaBH_4$ to give a single product, which was protected as acetate **47** in high overall yield. As **47** was equipped with no vinyl group that had been utilized for synthesis of α-hydroxy lactone in the previous syntheses of deoxy analogues of tetrodotoxin, new methodology for this purpose became necessary.[54] We initially planned to synthesize α-hydroxylactone such as **50** through lactone **49**, which would be obtained from **47**. Thus, deprotection of MMTr with TFA was followed by two step oxidation of the resulting alcohol with *o*-iodoxybenzoic acid (IBX) in DMSO[55] and then sodium chlorite to give lactone **49**. However, all attempts of hydroxylation at the α-position were unsuccessful. In the event, we fortunately found that heating aldehyde **48** with DBU in *o*-DCB at 130 °C provided cyclic vinyl ether **52** via *Z*-enolate **51**.[56] The selective formation of *Z*-enol (**51**) might be attributed to the abstraction of the axial proton at the C-9 position through a chelated intermediate as in Figure 5, and formation of the resulting cyclic vinyl ether **52** was under the stereoelectronic control by opening the quasi equatorial epoxide bond. It was then oxidized successively with $OsO_4$−NMO[57] and IBX[55] to give α-ketolactone **53** in 68% yield from **48**. Reduction of **53** with $NaBH_4$ furnished α-hydroxy-lactone **50** quantitatively as a single product.[58] The configuration of the C-9 position was confirmed by observing the long-range coupling between the H-9 and H-4a. The high stereoselectivity should be due to the severe steric hindrance of the axial acetoxy group at the C-5 position. The product **50** possesses a fully

(46) (a) Hirama, M.; Shigemoto, T.; Yamazaki, Y.; Ito, S. *J. Am. Chem. Soc.* **1985**, *107*, 1797−1798. (b) Hirama, M.; Ito, S. *Heterocycles* **1989**, *28*, 295−313.
(47) Einhorn, J.; Einhorn, C.; Ratajczak, F.; Pierre, J.-L. *J. Org. Chem.* **1996**, *61*, 7452−7454.
(48) (a) Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175−1176. (b) Kraus, G. A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825−4830. (c) Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888−890.
(49) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475−1478.
(50) Kocovsky, P. *Tetrahedron Lett.* **1986**, *27*, 5521−5524.
(51) In addition, the stereoelectronic effect (antiperiplanar effect) of the BOM protected hydroxyl group might operate to promote the conjugate addition.
(52) (a) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185−4188. (b) Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424−2426.
(53) (a) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1965**, *87*, 4214−4216. (b) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1967**, *89*, 1994, 2416−2423.
(54) The route through the intermediate bearing vinyl group at the C-8a position prepared from **44** was fruitless.
(55) (a) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272−7276. (b) Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019−8022.
(56) Preferential formation of *Z*-enolate **51** was supported by the following experiments; aldehyde **48** was treated with TIPS-OTf and DBU to give *Z*-alkenyl silyl ether, exclusively. The geometry was established by the coupling constant ($J$ = 7.5 Hz) of vinyl protons.
(57) VanRheenen, V.; Cha, D. Y.; Hartley, W. M. *Org. Synth. Coll.* **1988**, *6*, 342−348.
(58) For a similar synthesis of α-hydroxyl lactone, see: Corey, E. J.; Ghosh, A. K. *Tetrahedron Lett.* **1988**, *26*, 3205−3206.

***Scheme 9.*** Construction of the Bicyclic Compounds



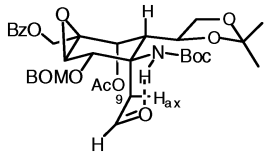



***Figure 5.*** Conformation of the enol precursor **48**.

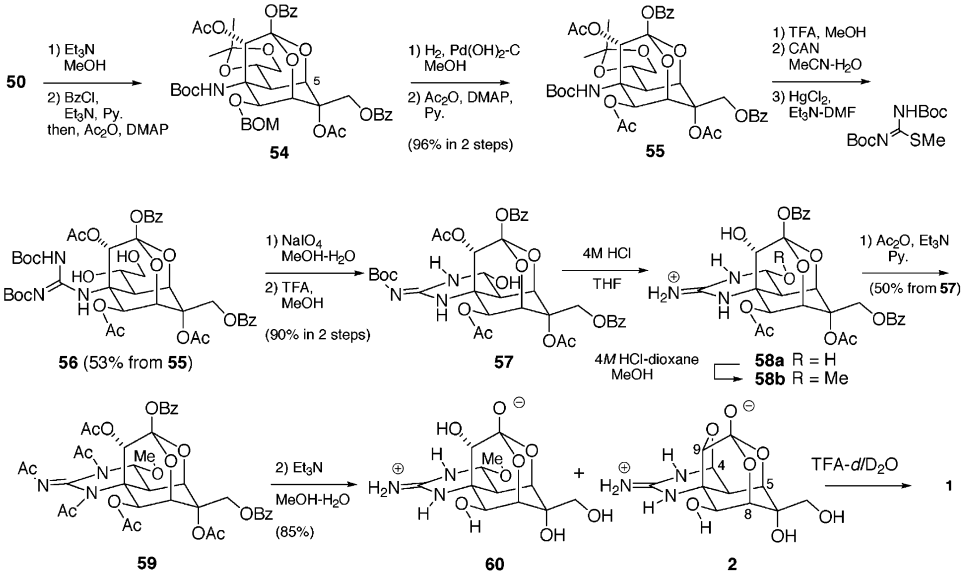
functionalized cyclohexane with the correct stereogenic centers for tetrodotoxin.

## Introduction of Guanidine and Completion of the Total Synthesis

We now focused on a final stage for the cyclic guanidine moiety, one of the unique structural features of tetrodotoxin. According to the synthetic plan, the lactone of **50** was protected as ortho ester with a hydroxyl group at the C-5 position (Scheme 10). Deacylation[59] of **50** was followed by benzoylation[60] and subsequent acetylation to provide ortho ester **54**. Prior to guanidinylation with sterically crowded di-Boc-protected isothiourea, the steric congestion was diminished around the amino group of **54** by transforming the BOM group to the corresponding acetate in two steps involving hydrogenolysis and acetylation.[61] Attempts to deprotect both the Boc and acetonide of **55** under conventional acidic conditions failed to give six-membered cyclic carbamate which formed by participation of the secondary hydroxyl group of the 1,2-diol to the carbonyl group of Boc. Extensive experiments led us to find that two step deprotection was necessary; thus, acid hydrolysis of the acetonide of **55** was followed by removal of the Boc group with ceric ammonium nitrate (CAN) in aqueous acetonitrile.[62] The resulting amine was directly treated with *N*,*N*′-diBoc-*S*-methylisothiourea[63] in the presence of mercuric chloride[64] to furnish **56** in good overall yield. Cleavage of the 1,2-diol with sodium periodate provided aldehyde,[65] which was further treated with methanolic TFA to give cyclic guanidine **57** with a hemiaminal structure.[66] The remaining Boc group was further deprotected with aqueous 4 M hydrochloric acid to give the desired cyclic guanidine **58a**.[67] To protect the labile hemiaminal moiety,[68] **58a** was treated with 4 M HCl−dioxane in methanol to give methyl aminal **58b**, which was isolated as the peracetate **59** in 50% overall yield from **57**. The product **57** was the last intermediate in the synthesis that could be readily purified in a preparative scale by silica gel chromatography. Toward the goal, all the acyl protective groups were removed with triethylamine in aqueous methanol to furnish a 4:1 mixture of 4-methoxytetrodotoxin (**60**) and 4,9-anhydro-4-*epi*tetrodotoxin (**2**)[69] in 85% yield. The $^1$H and $^{13}$C NMR spectra of **60** were fully assigned by HSQC,[70] while $^1$H NMR spectra of **2** were in good agreement with that of the natural product.[71] As transformation of these two tetrodotoxin derivatives to tetrodotoxin (**1**) under acidic conditions were reported,[5,6] a formal total synthesis of tetrodotoxin was completed at this stage. In our experiments, 4-methoxytetrodotoxin (**60**) was further treated with 2% TFA-*d* in deuterium oxide to give a mixture of tetrodotoxin (**1**) and 4,9-anhydrotetrodotoxin (**2**) in 65% and 15% yield, respectively. 4,9-Anhydrotetrodotoxin (**2**) was hydrolyzed under the same condition to furnish tetrodotoxin (**1**) in 63% yield along with a

(59) The reaction temperature below 15 °C was crucial to prevent from epimerization at the C-9 position.
(60) Triethylamine was required for benzoylation of the ortho ester. In the absence of the amine, only the primary alcohol at the C-11 position was benzoylated.
(61) Protection of the hydroxyl group at the C-8 position prevented from participation of the hydroxyl group to the proximate Boc group, leading to cyclic carbamate.
(62) Hwu, J. R.; Jain, M.; Tsay, S.-C.; Hakimelahi, G. H. *Tetrahedron Lett.* **1996**, *37*, 2035−2038.
(63) Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1987**, *52*, 1700−1703.
(64) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677−7680.
(65) The aldehyde was stable as anticipated. No $\beta$-elimination was observed under the conditions.
(66) The product **57** provided different $^1$H NMR spectra in each measurement, presumably because **57** might exist as a mixture of guanidine and guanidinium forms.
(67) Deprotection of all the acyl groups of **58a** proved very difficult; for example, aqueous ammonia in methanol caused complete decomposition, probably because of extreme instability of the hemiaminal moiety under basic conditions. For the stability of tetrodotoxin, see: Mosher, H. S. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 32−43.
(68) Nishikawa, T.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Tetrahedron* **2001**, *55*, 4325−4340.
(69) Upon exposure of **60** to the conditions, **2** was not detected.
(70) $^1$H NMR chart (60 MHz) of **60** was reported in ref 4; however, it is difficult to compare with that of the synthetic **60**, because the literature did not provide the values of the chemical shifts and coupling constants.
(71) Nakamura, M.; Yasumoto, T. *Toxicon* **1985**, *23*, 271−276.

***Scheme 10.*** Introduction of Guanidine and the Total Synthesis




small amount of recovered **2**. The mixture was purified with an ion exchange column by eluting with 0.05 N AcOH to give almost pure **1**, which was in slow exquilibrium to be purified. Synthetic tetrodotoxin was found to be identical with natural tetrodotoxin by comparison of spectroscopic data ($^1$H, $^{13}$C NMR,[72] and HR−FABMS), and specific rotation of the synthetic **1** was $[\alpha]^{28}_D$ +1 (*c* 0.08, 0.05 N AcOH−$H_2O$)(error being ± 2), which was nearly zero but different from the value $[\alpha]_D$ −8.64 (dissolved 855 mg of TTX in 10 mL of dil AcOH) reported in 1953.[73] It may be because of a different ratio of the components in the equilibrium mixture under different acid concentrations; thus, no practical component change was observed for a week at 0.05 N AcOH−$H_2O$. Remeasurement of the natural **1** showed $[\alpha]^{28}_D$ −3.0 (*c* 0.31, 0.05 N AcOH) after chromatographic separation from **2** with 15% of **3**. The rotation value of **1** is concluded to be nearly zero. Thus, we have concluded the first asymmetric total synthesis of tetrodotoxin.

## Conclusion

The first asymmetric total synthesis of tetrodotoxin (**1**) has been accomplished via a new route from 2-acetoxy-tri-*O*-acetyl-D-glucal as the chiral starting material. The synthesis first aimed at the construction of hydroxylated key cyclohexane derivatives through Sonogashira coupling, Claisen rearrangement, dipole-driven enolization and oxidation, and directed aldol condensation. Installation of the nitrogen atom was achieved through an intramolecular conjugate addition. The cage structure was constructed by the intramolecular *Z*-enol attack to the epoxide, and stereoselective oxidation provided the protected ortho ester structure. A guanidine group was introduced with retention of the solubility, and finally, a safer deprotection route provided tetrodotoxin.

**Acknowledgment.** This work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, PRESTO of Japan Science and Technology Corporation (JST), a Pfizer's Award in Synthetic Organic Chemistry, the Naito Foundation and the 21st Century COE grant. The authors thank JSPS for a DC scholarship and the Suzuki scholarship to N.O. Elemental analyses and measurements of 600 MHz NMR spectra were performed by Mr. S. Kitamura and Mr. K. Koga (analytical laboratory in this school), whom we thank. We also thank Prof. F. Horio (in this school) for help in the bioassay of synthetic tetrodotoxin.

**Supporting Information Available:** Experimental procedures and analytical and spectral characterization data for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA0342998

(72) For high-resolution NMR data of **1**, see: Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H. *J. Am. Chem. Soc.* **1988**, *110*, 2344−2345.

(73) The $[\alpha]_D$ value of natural **1** was reported to be −8.64 (*c* 8.55 in dilute AcOH) from very old literature, which was published before the structure elucidation (Tsuda, K.; Kawamura, M. *Pharm. Bull. Japan* **1953**, *1*, 112−113). The opposite sign may be due to component ratio differences in the equilibrium mixtures under different concentrations of **1** and acid, which is not clearly reported. As a reference, the plus sign of $[\alpha]_D$ was observed with that of 11-deoxytetrodotoxin (**2**); thus, the natural **2** lit.[19] $[\alpha]_D$ +5.37 (*c* 0.34, 0.05 N AcOH−$H_2O$); synthetic **2** lit.[14] $[\alpha]_D$ +13 (*c* 0.065, 0.05 N AcOH−$H_2O$). Synthetic **1** showed a fatal toxicity with a mouse (ip) test.