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Asymmetric Total Synthesis of 11-Deoxytetrodotoxin, a Naturally Occurring Congener

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Abstract: Tetrodotoxin, a toxic principle of puffer fish poisoning, is a specific blocker of sodium channel. Despite many synthetic efforts since the structure elucidation in 1964, the only total synthesis of the racemic tetrodotoxin has been reported by Kishi and co-workers. In the course of our studies directed toward the total synthesis to analyze biologically interesting issues associated with tetrodotoxin, we accomplished a highly stereocontrolled synthesis of (−)-5,11-dideoxytetrodotoxin in 1999. Based on the synthesis, we describe herein the first total synthesis of 11-deoxytetrodotoxin, a naturally occurring analogue. The synthesis started from an allylic alcohol, the same intermediate for the synthesis of 5,11-dideoxytetrodotoxin. Epoxidation of the allylic alcohol was followed by isomerization with Ti(*i*-PrO)$_4$ to give an α-hydroxy allylic alcohol, in which the configurations of the two hydroxyl groups were inverted by oxidation and then a 2-step reduction. Further epoxidation of the allylic alcohol and ozonolysis of the remaining vinyl group gave an aldehyde, which reacted with magnesium acetylide to give a propargyl alcohol in a stereoselective manner. Oxidative cleavage of the acetylenic moiety with RuO_4 afforded a fully functionalized lactone for 11-deoxytetrodotoxin. Crucial guanidinylation was achieved from trichloroacetamide according to our own method to give acetyldibenzylguanidine. Finally, deprotection of benzyl groups, acetates, and acetal furnished 11-deoxytetrodotoxin.

Introduction

Tetrodotoxin **1** (Figure 1), originally isolated from puffer fish, is one of the best-known marine natural products because of its novel chemical architecture and its potent biological activity, which can lead to fatal poisoning.[1] The structure of this compound was revealed by three groups, in 1964, including Hirata-Goto, Tsuda, and Woodward, to be an unprecedented dioxa-adamantane skeleton functionalized by hydroxy groups, an ortho ester showing acidity (pK_a = 8.7), and cyclic guanidine with hemiaminal.[2] The toxicity is attributed to a specific blockage of voltage-dependent sodium channels, a finding that has led to its use as an indispensable biochemical tool in the field of neurophysiology.[3] However, the detailed bound structure of tetrodotoxin to sodium-channel protein has not been solved despite many efforts.[4] Tetrodotoxin and its deoxy analogues such as **2**, **3**, and **4**[5] have been isolated not only from puffer fish, but also from many other animals such as newt, frog, octopus, and crab. These analogues are regarded as biosynthetic precursors or metabolites of tetrodotoxin, although the biosynthetic pathway has not been elucidated.[6] Cultured puffer fish were not toxic,[7]

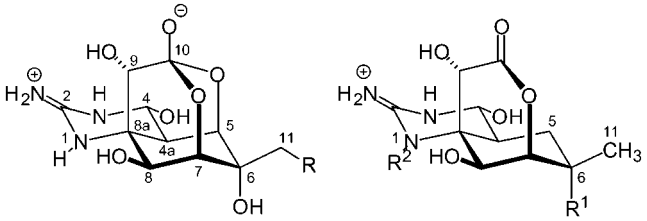

Figure 1. Structures of tetrodotoxin and its analogues.

tetrodotoxin-producing bacteria were isolated,[8] and tetrodotoxin was detected in many marine animals with a diet of puffer fish.[9] From these facts, it has been accepted that tetrodotoxin is

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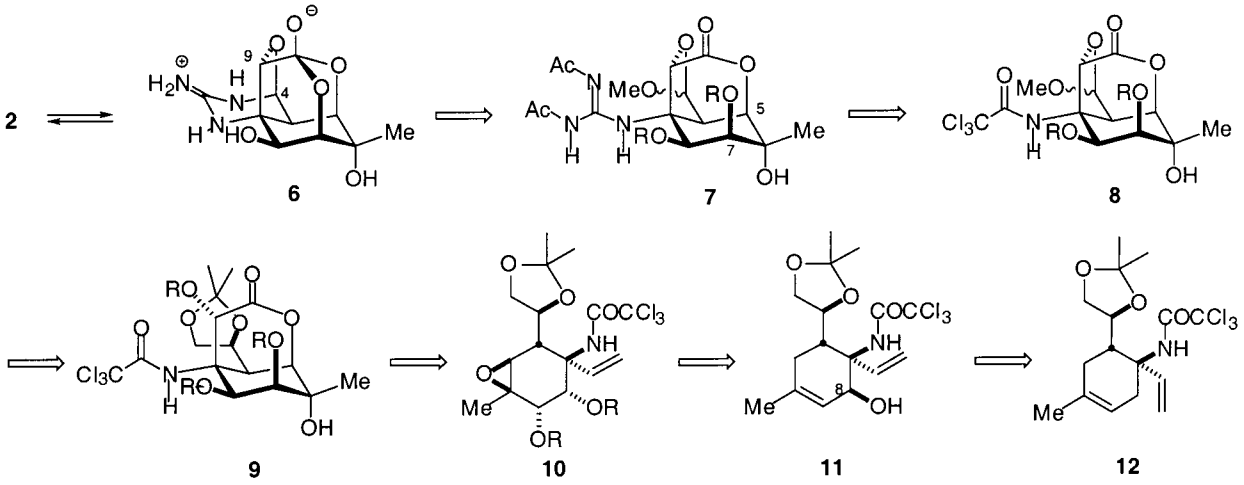
(1) Review: (a) Kao, C. Y., Lovinson, S., Eds. Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel. *Ann. N.Y. Acad. Sci.* **1986**, *479*. (b) Kobayashi, J.; Ishibashi, M. In *Comprehensive Natural Products Chemistry*; Pergamon: New York, 1999; Vol. 8, p 480.
(2) (a) Goto, T.; Kishi, Y.; Takahashi, S.; Hirata, Y. *Tetrahedron* **1965**, *21*, 2059. (b) Tsuda, K.; Ikuma, S.; Kawamura, M.; Tachikawa, R.; Sakai, K.; Tamura, C.; Amakasu, O. *Chem. Pharm. Bull*. **1964**, *12*, 1357. (c) Woodward, R. B. *Pure. Appl. Chem*. **1964**, *9*, 49.
(3) (a) Narahashi, T. *Physiol. Rev*. **1974**, *54*, 813. (b) Hucho, F. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 39.
(4) For example, (a) Nakayama, H.; Hatanaka, Y.; Takai, M.; Yoshida, E.; Kanaoka, Y. *Ann. N.Y. Acad. Sci.* **1993**, *707*, 349. (b) Noda, M.; Suzuki, H.; Numa. S.; Stuehmer, W. *FEBS Lett*. **1990**, *259*, 213. (c) Terlau, H.; Heinemann, S. H.; Stuehner, W.; Pusch, M.; Conti, F.; Imoto, K.; Numa, S. *FEBS Lett.* **1991**, *293*, 93. (d) Lipkind, G. M.; Fozzard, H. A. *Biophys. J.* **1994**, *66*, 1. (e) Recently, the gross 3D structure at 19 Å was determined. See: Sato, C.; Ueno, Y.; Asai, K.; Takahashi, K.; Sato, M.; Engel, A.; Fujiyoshi, Y. *Nature* **2001**, *409*, 1047.
(5) For a review, see: Yotsu-Yamashita, M. *J. Toxicol. Toxin Rev.* **2001**, *20*, 51.
(6) Shimizu, Y.; Kobayashi, M. *Chem. Pharm. Bull*. **1983**, *31*, 3625.
(7) Shimizu, U.; Matsui, H.; Sato, H.; Yamamori, K. *Mar. Sci. Mon*. **1984**, *16*, 560 (in Japanese).
(8) (a) Yasumoto, T.; Yamamura, D.; Yotsu, M.; Michishita, T.; Endo, A.; Kotaki, Y. *Agric. Biol. Chem*. **1986**, *50*, 793. (b) Noguchi, T.; Jeon, J.-K.; Arakawa, O.; Sugita, H.; Deguchi, Y.; Shida, Y.; Hashimoto, K. *J. Biochem*. **1986**, 99, 311. (c) Shimizu, Y. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 24.
(9) Yasumoto, T.; Nagai, H.; Yasumura, D.; Michishita, T.; Endo, A.; Yotsu, M.; Kotaki, Y. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 44.

Scheme 1. Synthetic Plan for 11-Deoxytetrodotoxin



accumulated through the food chain. Recently tetrodotoxin-binding proteins were isolated from puffer fish,[10] and these proteins might play some role in the specific accumulation and the resistance against tetrodotoxin.[11] Actual biological functions of the toxin also have been of significant interest.[12]

To elucidate the above problems on a molecular level, a supply of suitably labeled tetrodotoxin derivatives has been highly desired by means of total synthesis, because derivatization of natural tetrodotoxin is extremely difficult.[13] Despite many synthetic efforts,[14] however, the only total synthesis of (±)-tetrodotoxin **1** was achieved by Kishi and co-workers in 1972.[15] Our continuous efforts directed toward the total synthesis culminated in achieving a stereocontrolled synthesis of a chiral (−)-5,11-dideoxytetrodotoxin (**5**) in 1999[16] as the first asymmetric synthesis among tetrodotoxin analogues. However, the synthetic **5** showed little biological activity (toxicity toward mice and affinity to Na channel proteins),[17] which prompted us to synthesize biologically active tetrodotoxin analogues having the characteristic ortho ester group. Herein, we describe the first total synthesis of 11-deoxytetrodotoxin (**2**), a naturally occurring as well as biologically active tetrodotoxin analogue isolated from puffer fish and newts by Yasumoto and co-workers.[18]

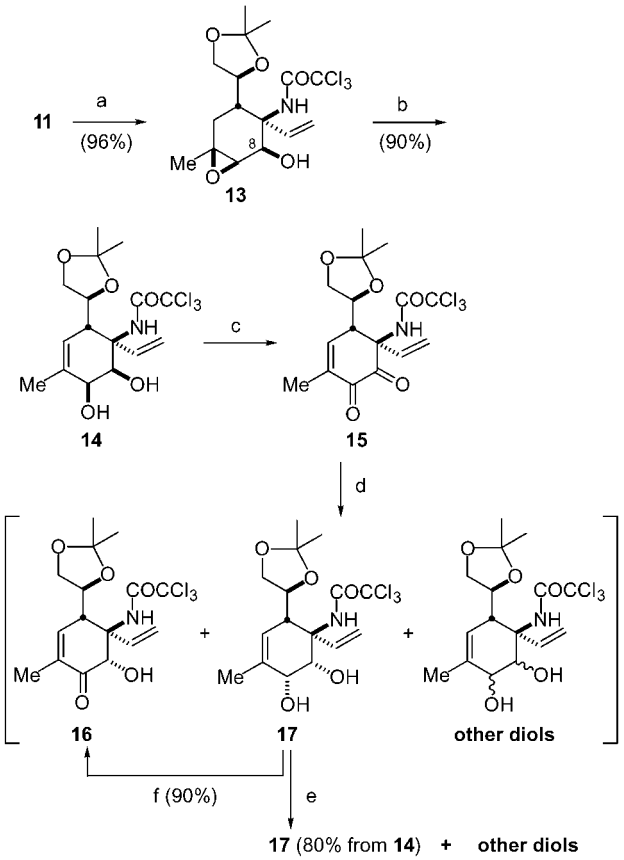
Results and Discussion

Synthetic Plan.[19] Our retrosynthetic plan for 11-deoxytetrodotoxin (**2**) is shown in Scheme 1, based on the synthesis of (−)-5,11-dideoxytetrodotoxin (**5**). 11-Deoxytetrodotoxin (**2**) and 4,9-anhydro-11-deoxytetrodotoxin (**6**) are interconvertible under acidic conditions.[2] The ortho ester was synthetically equivalent to δ-hydroxy lactone. As the 4,9-anhydro structure played a crucial role in the protection of the unstable C-9 hydroxyl group under gunanidinylation[15] in the synthesis of **5**, we planned to use a lactone intermediate bearing the 4,9-anhydro structure for the synthesis of **2**. When an aldehyde was generated at the C-4 position, facile β-elimination of the hydroxyl group at C-5 was anticipated, because of the diaxial relationship between the proton at C-4a and the hydroxy group at C-5.[20] Consequently, the lactone **7** at C-5 was chosen from the two possible lactone candidates at the C-5 and C-7 positions, because the lactone ring formation in **7** would prevent the β-elimination.[21] The guanidine group could be prepared from the trichloroacetamide **8** according to the guanidine synthesis developed in this laboratory.[22] The anhydro moiety of **8** would be prepared from the lactone-acetonide **9** in an analogous way described in the synthesis of 5,11-dideoxytetrodotoxin (**5**). The lactone **9** was then retrosynthesized into vinylepoxide **10**, which was envisaged to synthesize from the same intermediate **11** as that of **5**. Allylic alcohol **11** was readily prepared from **12**, the common synthetic intermediate for various tetrodotoxin analogues in our studies.[23]

Hydroxylation of the Cyclohexane Ring. The synthesis commenced with epoxidation of allylic alcohol **11** with *m*-chloroperbenzoic acid (MCPBA) to give exclusively β-epoxide **13** (Scheme 2). This selectivity is due to steric hindrance of the axially oriented vinyl group in the cyclohexane ring as well

(10) (a) Matsui, T.; Yamamori, K.; Furukawa, K.; Kono, M. *Toxicon* **2000**, *38*, 463. (b) Yotsu-Yamashita, M.; Sugimoto, A.; Terakawa, T.; Shoji, Y.; Miyazawa, T.; Yasumoto, T. *Eur. J. Biochem.* **2001**, *268*, 5937.
(11) (a) Shahjahan, M.; Yamada, M.; Nagaya, M.; Kawai, M.; Nakazawa, A. *Ann. N.Y. Acad. Sci*. **1993**, *707*, 346. (b) Yotsu-Yamashita, M.; Nishimori, K.; Nitanai, Y.; Isemura, M.; Sugimoto A.; Yasumoto, T. *Biochem. Biophys. Res. Commun*. **2000**, *267*, 403.
(12) (a) Saito, T.; Noguchi, T.; Harada, T.; Murata, O.; Hashimoto, K. *Bull. Jpn. Soc. Sci. Fish* **1985**, *51*, 1175. (b) Matsumura, K. *Nature* **1995**, *378*, 563.
(13) For example, Mosher, H. S. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 32.
(14) For leading references from other laboratories, see: (a) Noya, B.; Paredes, M. D.; Ozores, L.; Alonso, R. *J. Org. Chem.* **2000**, *65*, 5960. (b) Burgey, C. S.; Vollerthun, R.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 1609. (c) Sato, K.; Kajihara, Y.; Nakamura, Y.; Yoshimura, J. *Chem. Lett.* **1991**, 1559. (d) Nachman, R. J.; Hönel, M.; Williams, T. M.; Halaska, R. C.; Mosher, H. S. *J. Org. Chem.* **1986**, *51*, 4802. (e) 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, 3627. (f) Speslacis, J. Ph.D. Thesis, Harvard University, 1975.
(15) (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. (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.
(16) (a) Nishikawa, T.; Asai, M.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 3081. (b) Asai, M.; Nishikawa, T.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Tetrahedron* **2001**, *57*, 4543.
(17) Yotsu-Yamashita, M.; Nishikawa, T.; Asai, M.; Isobe, M. Unpublished results.
(18) Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, M. *J. Am. Chem. Soc.* **1988**, *110*, 2344.
(19) The numbering used in this paper corresponds to that of tetrodotoxin.
(20) Kishi and co-workers encountered the serious problem of β-elimination. See ref 15.
(21) A lactone at the C-5 hydroxy group was synthesized by Kishi and co-workers to prevent the β-elimination in their synthesis of (±)-tetrodotoxin. See: (a) Kishi, Y. *J. Synth. Org. Chem. Jpn*. **1974**, *32*, 855 (in Japanese). (b) Aratani, M.; Fukuyama, T.; Tanino, H.; Kishi, Y.; Mataura, T.; Kakoi, H.; Inoue, S. Annual Meeting of Japan Chemical Society, 1974; abstract paper III, p 1427.
(22) (a) Yamamoto, N.; Isobe, M. *Chem. Lett.* **1994**, 2299. (b) Nishikawa, T.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Tetrahedron* **1999**, *55*, 4325.
(23) Nishikawa, T.; Asai, M.; Ohyabu, N.; Yamamoto, N.; Fukuda, Y.; Isobe, M. *Tetrahedron* **2001**, *57*, 3875.

Scheme 2 [a]



[a] Conditions: (a) MCPBA, Na_2HPO_4, CH_2Cl_2. (b) Ti(O*i*-Pr)$_4$, $(CH_2Cl)_2$, reflux. (c) IBX (4 equiv), DMSO, 60 °C. (d) LiAlH(O*t*-Bu)$_3$, LiBr, THF, −78 °C. (e) $NaBH_4$, $CeCl_3(H_2O)_7$, MeOH, −78 °C. (f) IBX (1.5 equiv), DMSO, room temperature.

as the directed effect of the β-hydroxyl group at C-8. Lewis acid-mediated isomerization of epoxide **13** to **14** was then examined, since it was likely that a conventional strong base such as lithium amide was incompatible with the base-labile trichloroacetamide group.[24] Al(*i*-PrO)$_3$[25] was used to isomerize **13** to a 4:1 mixture of the desired *endo*-allylic alcohol **14** and the undesired *exo*-allylic alcohol. In contrast, **13** was treated with Ti(*i*-PrO)$_4$[26] in dichloroethane at the reflux temperature to afford **14** as a single product in about 90% yield. The high regioselectivity was attributed to coordination of the titanium ion to both the epoxide oxygen and hydroxy group followed by epoxide ring cleavage, as suggested by Sharpless.[26] Both configurations of the resulting diol **14** would be inverted at the same time by the oxidation−reduction sequence, utilizing the preferential approach of reagents from the less hindered β-face of these intermediates. Oxidation of the diol **14** was best accomplished with IBX (*o*-iodoxybenzoic acid)[27] to give diketone **15**, which was successively reduced in the next reduction without purification.[28] We expected that stereoselective reduction of diketone **15** would be easily realized, because steric hindrance of the axially oriented vinyl group had been employed as an important control element in many stereoselective reactions

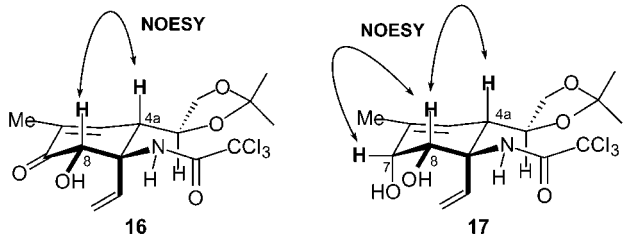


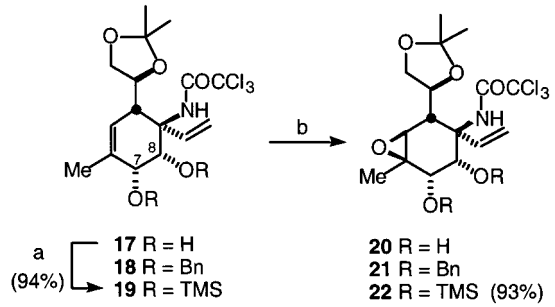
Figure 2. Conformation of **16** and **17**.

on the cyclohexane ring.[16,22,29] However, stereoselective reduction of the diketone **15** proved to be quite difficult. Reduction with a variety of hydrides gave a mixture of all four possible diol products with poor selectivities. Fortunately, much experimentation led us to find that the two-step reduction involving LiAlH(*t*-BuO)$_3$ in the presence of LiBr[30] followed by exposure to $NaBH_4$-$CeCl_3$[31] afforded diol **17** with the desired configuration in 80% isolated yield from **14**. The first reduction gave a mixture of α-hydroxyketone **16** and the desired diol **17** in a ratio of 3:1.[32] Since the subsequent Luche reduction of the crude products[30] gave **17** in good isolated yield, the reduction of **16** should proceed in high diastereoselectivity. In fact, **16** prepared from oxidation of diol **17** with IBX (1.5 equiv) was treated under Luche conditions at −78 °C to afford largely diol **17**. Interestingly, an excess of the first reducing agent LiAlH(*t*-BuO)$_3$ did not complete the reaction, while reduction of diketone **15** under Luche conditions gave a mixture of diols in a significantly decreased stereoselectivity. The configurations of the newly generated asymmetric centers of **16** and **17** were assigned on the basis of NOESY spectra as shown in Figure 2. Fortunately, only the desired diol **17** was easily separated by silica gel column chromatography, while a mixture of the undesired other diols could be transformed to diketone **15** through re-oxidation with IBX as described above.

Further functionalization of diol **17** also proved to be problematic. We initially planned to protect two hydroxyl groups at the C-7 and -8 position as benzyl ether, because the benzyl groups could be concomitantly removed in the deprotection of dibenzylguanidine at the last stage of the synthesis. However, benzylation [NaH, benzyl bromide in DMF-THF] of the two hydroxyl groups gave dibenzyl ether **18** in a very poor yield (23%) along with monobenzyl ethers as byproducts.[33] Furthermore, attempted epoxidation of the resulting dibenzyl ether **18** with MCPBA did not proceed even under forcing conditions at dichloroethane reflux temperature in the presence of Kishi's radical inhibitor[34] and with dimethyldioxirane[35] in acetone.[36] We then examined the trimethylsilyl (TMS) protecting group

(24) Trichloroacetamide produced isocyanate under basic conditions, see: Atanassova, I. A.; Petrov, J. S.; Mollov, N. M. *Synthesis* **1987**, 734.
(25) (a) Eschinasi, E. H. *J. Org. Chem.* **1970**, *35*, 1598. (b) Terao, S.; Kato, K. *Synthesis* **1987**, 467.
(26) Morgans, D. J., Jr.; Sharpless, K. B.; Traynpor, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 462.
(27) Frigerio, N.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019.
(28) No enolization of **15** was observed by ^{1}H NMR spectra of the crude product. Since enolization of the diketone **15** was our major concern, we initially attempted the reduction of epoxydiketone that was prepared from epoxidation of **14** with MCPBA followed by oxidation of 1,2-diol (Ac_2O-DMSO). However, little selectivity was observed in the reduction under a variety of conditions.
(29) Yamamoto, N.; Nishikawa, T.; Isobe, M. *Synlett* **1995**, 505.
(30) Mori, Y.; Suzuki, M. *Tetrahedron Lett.* **1989**, *30*, 4383.
(31) Luche, J. L.; Gamal, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 5848.
(32) A small amount (less than 5%) of the undesired diols was also observed by ^{1}H NMR; however, the stereochemistries have not been determined.
(33) Other benzylation conditions with benzyl trichloroacetimidate in the presence of acid failed. Wessel, H.-P.; Iversen, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1989**, *54*, 465.
(34) Kishi, Y.; Aratani, M.; Tanino, H.; Fukuyama, T.; Goto, T.; Inoue, S.; Sugiura, S.; Kakoi, H. *J. Chem. Soc., Chem. Commun.* **1972**, 64.
(35) (a) Murray, R. W. *Chem. Rev.* **1989**, *89*, 1187. (b) Adam, W.; Edwards, J. O. *Acc. Chem. Res.* **1989**, *22*, 205.

Scheme 3 [a]



[a] Conditions: (a) TMSOTf, Py, MeCN. (b) MCPBA, Na_2HPO_4, CH_2Cl_2, room temperature.

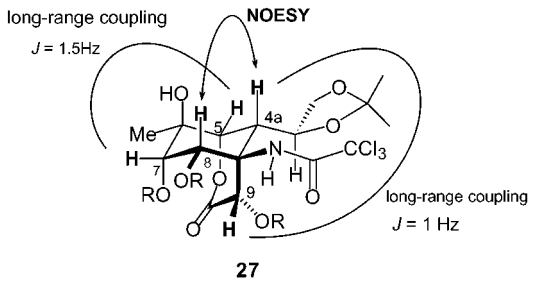
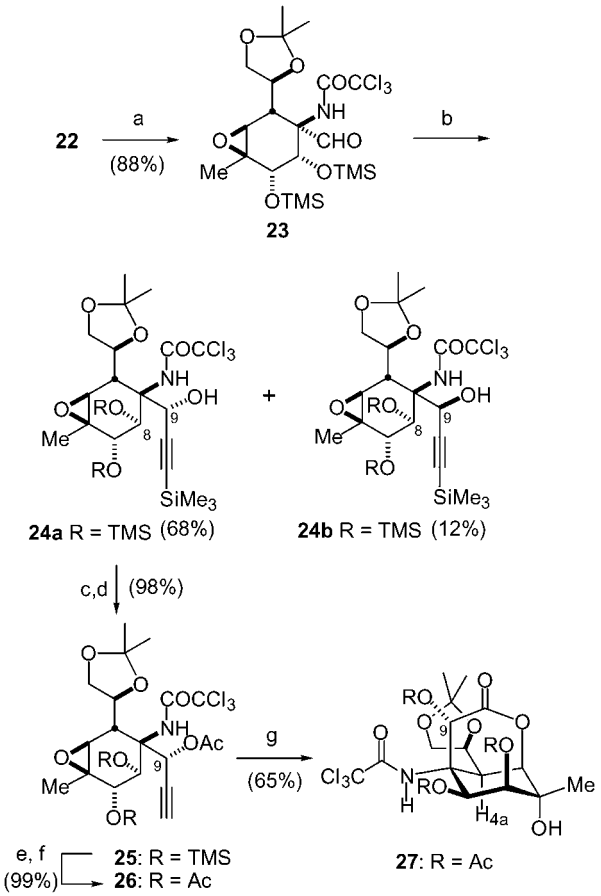


Figure 3.

that also could be removed at the last stage of the synthesis. Although the conventional conditions (TMSCl, imidazole, DMF or TMSOTf, 2,6-lutidine, CH_2Cl_2) for silylation were not applicable to **17**, we fortunately found that the conditions (TMSOTf, pyridine, CH_3CN) described by Heathcock[37] gave the desired bis-TMS protected diol **19** in high yield. In sharp contrast to dibenzyl ether **18**, **19** was smoothly epoxidized with MCPBA *at room temperature* to give **22** as a single product in high yield.[38,39]

Synthesis of Lactone Intermediate. With multigram quantities of **22** in hand, the vinyl group was transformed into α-hydroxycarboxylic acid according to the methods for (−)-5,11-dideoxytetrodotoxin. The aldehyde **23** obtained by ozonolysis of **22** was treated with magnesium acetylide in THF to give a 5.5:1 separable diastereomeric mixture in favor of the desired isomer **24a** (Scheme 4),[40] while the reaction with lithium acetylide in THF gave a lower selectivity (1.7:1).[41] On the basis of the proposed mechanism of stereoselective addition of the acetylide to the aldehyde in the synthesis of **5**, we assumed that the configuration of the C-9 position of the major product **24a** would be the same as that of 11-deoxytetrodotoxin, although

Scheme 4 [a]

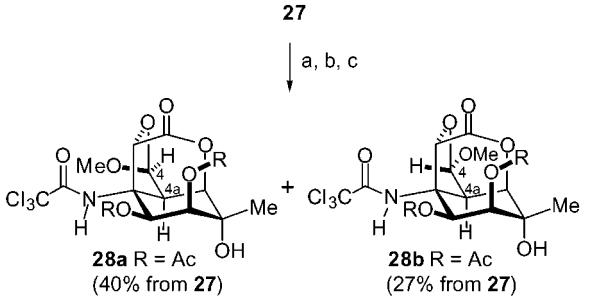


[a] Conditions: (a) O_3, MeOH, −78 °C; Me_2S. (b) TMS−C≡C−MgBr, THF, 0 °C to room temperature. (c) Ac_2O, Py. (d) *n*-Bu_4NF, MeOH, THF. (e) HF-Py, THF. (f) Ac_2O, Py. (g) $RuO_2(H_2O)_x$, $NaIO_4$, CCl_4, MeCN, H_2O.

the configuration could not be determined at this stage. The propargyl alcohol **24a** was transformed to propargyl acetate **25** in two steps involving acetylation followed by selective desilylation of silylacetylene with *n*-Bu_4NF in the presence of MeOH in THF.[42] Surprisingly, the acetylenic moiety of **25** was completely inert to RuO_4 oxidation,[43] probably due to steric hindrance of the two TMS ethers and trichloroacetamide. Thus, the two TMS ethers of **25** were converted to acetate's smaller protective group in two steps: (i) HF/Py, and (ii) Ac_2O/Py.[44] In this case, the acetylenic group of **26** was successfully cleaved with RuO_4,[43] and the resulting carboxylic acid intermediate spontaneously opened the epoxide under these conditions to furnish lactone **27** in good overall yield.[45] The structure of **27** was confirmed by extensive ^1H NMR analysis (Figure 3). The configuration of the C-9 position that was generated by the addition of acetylide to the aldehyde **23** was confirmed by observing the W-shaped long-range coupling ($J = 1$ Hz) between H-4a and H-9. Long-range coupling ($J = 1.5$ Hz) observed between H-5 and H-7 and NOESY correlation between H-4a and H-8 confirmed the configurations of the two hydroxyl groups as shown in Figure 3. Thus lactone **27** constitutes a fully

(36) Epoxidation of **17** with MCPBA also did not proceed at room temperature, while the epoxidation in the presence of Kishi's radical inhibitor at elevated temperature (dichloroethane reflux) gave **20** in only 52% yield.
(37) Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pilli, R.; Badertscher, U. *J. Org. Chem.* **1985**, *50*, 2095.
(38) One plausible explanation for the different reactivity might be the difference in the conformation of these substrates **18** and **19**. However, we could not find a difference in the coupling constants between protons at C-7 and C-8 of **18** and **19** (both 3.5 Hz).
(39) The stereochemistry of the epoxide could not be determined at this stage, although the structure was assumed to be *β* from the steric hindrance of the allylic siloxy group. The structure was finally determined by NMR experiments of lactone **27** derived from **22** (vide infra).
(40) The slightly better diastereomeric ratio (6:1) was obtained by titanium triisopropoxy acetylide in THF, but the reaction was sluggish (for 3 days at 5 °C). For titanium acetylide, see: (a) Tabusa, F.; Yamada, T.; Suzuki, K.; Mukaiyama T. *Chem. Lett.* **1984**, 405. (b) Krause, N.; Seebach, D. *Chem. Ber.* **1987**, *120*, 1845.
(41) Interestingly, the same reaction in diethyl ether as a solvent gave the reverse selectivity (**24a**/**24b** = 1:3.5 by ^1H NMR).
(42) Nishikawa, T.; Ino, A.; Isobe, M. *Tetrahedron* **1994**, *50*, 1449.
(43) Carisen, P. H.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(44) Attempted desilylation of all the TMS groups of **24a** in one operation with *n*-Bu_4NF in THF in the absence of MeOH gave not triol but a cyclic carbamate that was formed by attack of alkoxide at the C-8 of trichloroacetamide.
(45) Spontaneous lactonization may be due to the special nature of such a caged molecule. For other examples from our laboratory, see ref 29.

Scheme 5 [a]



[a] Conditions: (a) KCN, EtOH. (b) $HIO_4(H_2O)_2$, AcOMe. (c) *p*-TsOH, $CH(OMe)_3$, MeOH.

functionalized cyclohexane derivative for the synthesis of 11-deoxytetrodotoxin.

Installation of Guanidine and Completion of the Total Synthesis. In the synthesis of 5,11-dideoxytetrodotoxin, installation of the guanidine functionality was the most difficult task due to the hydroxy group instability at the C-9 position and the severe steric hindrance around the nitrogen-containing functionality.[16] As such, the hydroxyl group at the C-9 position was protected as an intramolecular mixed acetal with the aldehyde at the C-4 position. This protection proved to be critical not only during the installation of the guanidine, but also for secure deprotection at the final stages of the synthesis. Thus, the lactone **27** was converted to a 1.5:1 diastereomeric mixture of acetal **28a** and **28b**[46] in three steps involving selective deacetylation at the C-9 position,[47] oxidative cleavage of the acetonide,[48] and acetalization (Scheme 5). After separation with silica gel chromatography, the minor isomer **28b** was acetylated, and then subjected to the guanidinylation developed in this laboratory.[23] Thus, the trichloroacetamide (**28b**) was transformed to benzylurea (**29**), which was dehydrated under the conditions reported by Appel[49] to give a benzylcarbodiimide **30**. The addition of benzylamine was best accomplished with benzylamine hydrochloride under pyridine reflux conditions[16,22] to afford the dibenzylguanidinium salt, which was subsequently acetylated to give acetyldibenzylguanidine (**31**) in good overall yield. On the otherhand, the major isomer **28a** could be transformed to the corresponding carbodiimide in the same way as shown in Scheme 6. However, the addition of benzylamine to the carbodiimide derived from the major isomer **28a** under the same conditions gave the corresponding guanidine in poor yield, probably because of steric hindrance of the methoxy group at the acetal position. Thus, **28a** could be guanidinylated through the minor isomer **28b** obtainable by equilibration of **28a** under the acidic conditions (*p*-TsOH, $CH(OMe)_3$, MeOH).

The synthesis was completed by the successive removal of three types of protecting group as follows. (i) The benzyl groups of guanidine (**31**) were hydrogenolyzed in acetic anhydride to give diacetylguanidine (**32**); (ii) all the acetates were removed with aqueous ammonia; and (iii) the acetal was hydrolyzed with aqueous TFA. The crude products were separated by HPLC on an ion-exchange resin to give 11-deoxytetrodotoxin (**2**) and anhydro-11-deoxytetrodotoxin (**6**) in 29 and 55% yields, respectively.[18] These products **2** and **6** are interconvertible in acidic media. For example, a solution of anhydro-11-deoxytetrodotoxin (**6**) dissolved in 1% TFA-*d* in D_2O was kept at room temperature for 7 days to attain the equilibrium composed of **2**, **6**, and 4-*epi*-11-deoxytetrodotoxin in an ca. 5:1:1 ratio (from ^1H NMR). The ^1H and ^{13}C NMR spectra of both products **2** and **6** were identical with those of the natural products.[18]

Conclusion

In summary, we have achieved total synthesis of naturally occurring 11-deoxytetrodotoxin in an enantiomerically pure form. This work opens up the possibility of preparing suitable probes to elucidate related biological issues. Further synthetic studies toward tetrodotoxin and unnatural tetrodotoxin analogues are currently under investigation in our laboratory.

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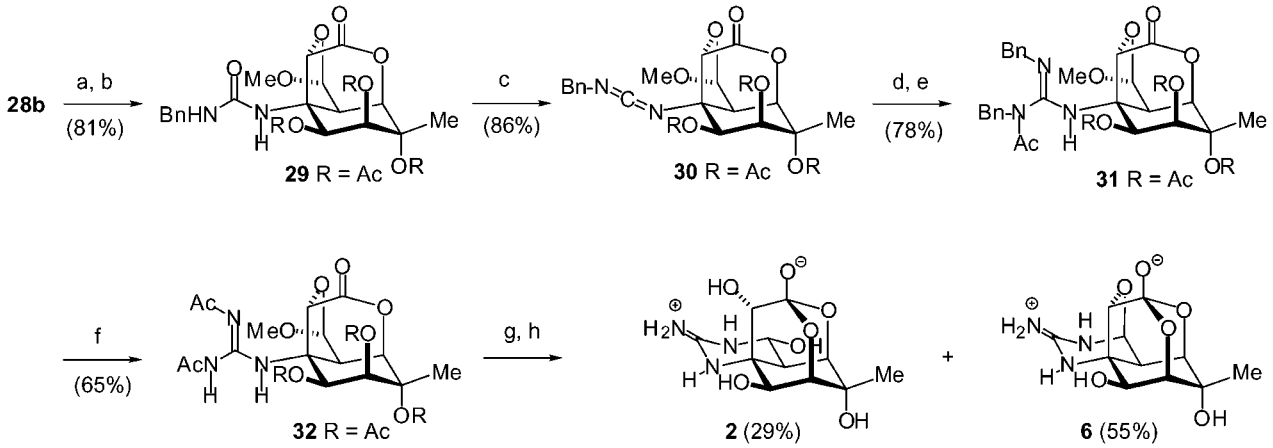
Scheme 6 [a]



[a] Conditions: (a) Ac_2O, DMAP, Py. (b) $BnNH_2$, Na_2CO_3, DMF, reflux. (c) Ph_3P, CBr_4, Et_3N, CH_2Cl_2. (d) $BnNH_2$·HCl, Py, reflux. (e) Ac_2O, Et_3N, Py. (f) H_2, $Pd(OH)_2$-C, Ac_2O. (g) NH_4OH, MeOH, H_2O. (h) TFA, H_2O.

Supporting Information Available: Full experimental details and spectroscopic data including NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

(46) The configurations of the acetal carbons (C-4) of **28a** and **28b** were determined from coupling constants between H-4 and H-4a (0 and 4 Hz, respectively). The dihedral angle between H-4 and H-4a of **28a** is almost 90°. The observed coupling constants are also in good agreement with calculated values (0.9 and 6.3 Hz) by MacroModel (MM2*).

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