ORGANIC LETTERS

2002
Vol. 4, No. 16
2679−2682

Stereocontrolled Synthesis of 8,11-Dideoxytetrodotoxin, Unnatural Analogue of Puffer Fish Toxin

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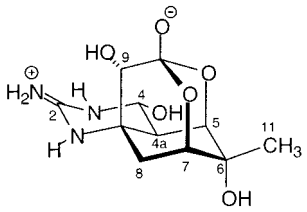
Received May 13, 2002

ABSTRACT



8,11-Dideoxytetrodotoxin, an unnatural tetrodotoxin analogue, was synthesized in a highly stereoselective manner from a common intermediate in our synthetic studies on tetrodotoxin. The synthesis features neighboring group participation of trichloroacetamide for stereoselective hydroxylation, protection of ortho ester, and guanidine installation with Boc-protected isothiourea.

Tetrodotoxin **1** (Figure 1),[1] originally isolated as a toxic principle of puffer fish poisoning, is one of the most famous and important marine natural products because of its novel structure and potent biological activity.[2] Since the mechanism of action of this molecule was revealed to be the specific blockage of voltage-dependent sodium channels that are responsible for nerve and muscle excitability, the toxin has been widely employed as an indispensable biochemical tool for neurophysiological study.[3] However, the detail of the bound structure has not been described to date despite many efforts such as photoaffinity labeling and site-directed mutagenesis;[4] severe limitations regarding the structural modifications needed for preparing potent probe molecules and the lack of the tertiary structure of the sodium channel protein have hindered elucidation of the bound structure of this toxin.[5,6] In the course of our synthetic studies on

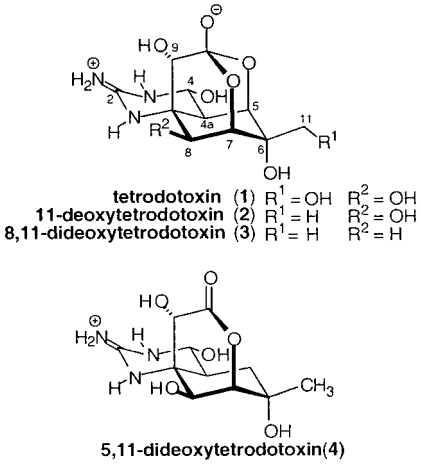


Figure 1. Structures of tetrodotoxin and its analogues.

(1) (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.

(2) Review: (a) Kao, C. Y.; Lovinson, S., *Ann. N.Y. Acad. Sci.* **1986**, *479*, 1. (b) Kobayashi, J.; Ishibashi, M. In *Comprehensive Natural Products Chemistry;* Pergamon: 1999; p 480.

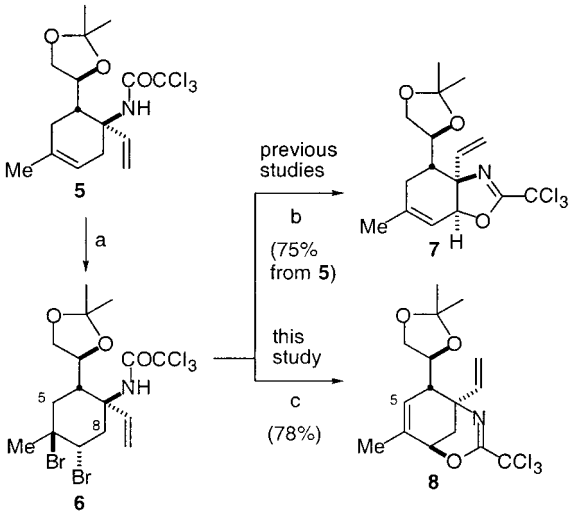
(3) (a) Narahashi, T. *Physiol. Rev.* **1974**, *54*, 813. (b) Hucho, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 39. (c) Anger, T.; Madge, D. J.; Mulla, M.; Riddall. D. *J. Med. Chem.* **2001**, *44*, 115.

10.1021/ol026177a CCC: $22.00
Published on Web 07/18/2002

tetrodotoxin, we succeeded in the asymmetric syntheses of 5,11-dideoxytetrodotoxin **4**[7] and 11-deoxytetrodotoxin **2**.[8−10] In this letter, we describe an efficient synthesis of 8,11-dideoxytetrodotoxin **3**, which was extremely difficult to prepare from naturally occurring tetrodotoxin. This study might shed light on the role played by the 8-hydroxyl group in the toxicity of the tetrodotoxin family.[11]

On the basis of the successful syntheses of 5,11-dideoxytetrodotoxin **4** and 11-deoxytetrodotoxin **2**, the synthesis started with dibromide **6**, which was readily prepared by bromination of the common key intermediate **5**, as described in our previous studies (Scheme 1).[12] In the previous

Scheme 1[a]

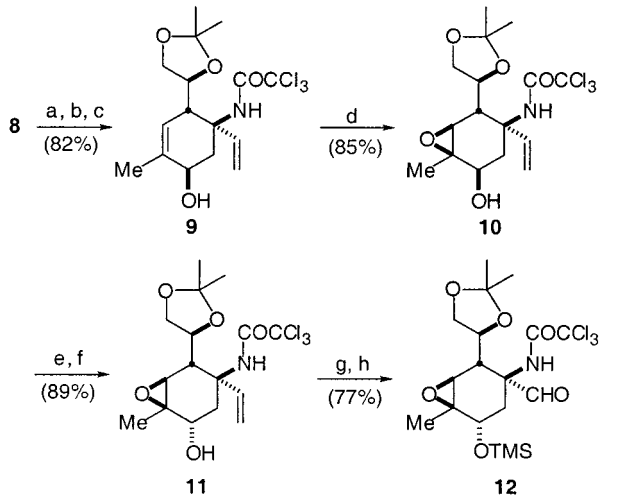


[a] Conditions: (a) PyH·Br_3, K_2CO_3, CH_2Cl_2, 10 °C. (b) DBU, DMF, rt. (c) K_2CO_3, MeOH, rt.

syntheses of **2** and **4**, hydroxylation at the C-8 position of **6** was accomplished by a novel neighboring group participation of trichloroacetamide with DBU in DMF to give oxazoline **7**, which was hydrolyzed under mild acidic conditions to afford an allylic alcohol at the C-8 position.[7] On the other hand, we found that the same dibromide **6** was treated with K_2CO_3 in MeOH to give a bicyclic iminoether **8** in good yield. The oxidation state of **8** has been set for 8,11-dideoxytetrodotoxin **3**.

The cyclic iminoether **8** was transformed to allylic alcohol **9** in good overall yield through three steps: (i) acid hydrolysis of iminoether **8**,[13] (ii) trichloroacetylation of the resulting amino alcohol, and (iii) methanolysis of trichloroacetate (Scheme 2). Epoxidation of allylic alcohol **9** with

Scheme 2[a]



[a] Conditions: (a) AcOH, THF, H_2O, rt. (b) CCl_3COCCl, Py, rt. (c) K_2CO_3, MeOH, rt. (d) MCPBA, Na_2HPO_4, CH_2Cl_2, rt. (e) PCC, 4 Å MS, CH_2Cl_2, rt. (f) $NaBH_4$, MeOH, 5 °C. (g) TMSCl, Et_3N, THF, rt. (h) O_3, CH_2Cl_2, −78 °C; Et_3N.

MCPBA gave β-epoxide **10** in 85% yield. The configuration of the C-7 position was inverted by oxidation with PCC and subsequent reduction with $NaBH_4$ to give α-alcohol **11** in 89% overall yield from **10**. The resulting alcohol **11** was protected as TMS ether.[14] The remaining vinyl group was then ozonized upon treatment of Et_3N as a reductant[15] to give unstable aldehyde **12**.

The epoxyaldehyde **12** was transformed to lactone **15**, as shown in Scheme 3. The aldehyde **12** reacted with lithium trimethylsilylacetylide to give propargyl alcohol **13** as a single stereoisomer.[16] As expected, the configuration of the

(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.

(5) Catterall, W. A. *Neuron* **2000**, *26*, 13.

(6) Recently, the gross three-dimensional structure at 19 Å was reported. See: Sato, C.; Ueno, Y.; Asai, K.; Takahashi, K.; Sato, M.; Engel, A.; Fujiyoshi, Y. *Nature* **2001**, *409*, 1047.

(7) (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.

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

(9) A single total synthesis of the racemate was reported. See: (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.

(10) 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, Cambridge, MA, 1975.

(11) For a review on tetrodotoxin analogues, see: Yotsu-Yamashita, M. *J. Toxicol. Toxin Rev.* **2001**, *20*, 51.

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

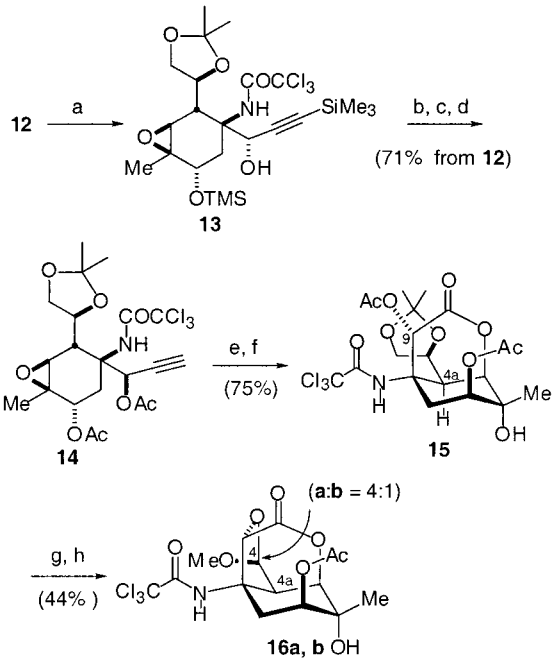
(13) Hydrolysis of **7** under acidic conditions gave allylic alcohol bearing trichloroacetamide,[7] while hydrolysis of **8** under the same conditions did not proceed. Attempted one-step conversion of **8** to **9** under forcing hydrolytic conditions failed.

(14) The protective group was critical not only for the further functionalization, but for final successful deprotection.

(15) (a) Isobe, M.; Iio, H.; Kawai, T.; Goto, T. *Tetrahedron Lett.* **1977**, 703. (b) Iio, H.; Isobe, M.; Kawai, T.; Goto, T. *Tetrahedron* **1979**, *35*, 941.

(16) Use of magnesium acetylide in THF gave a decreased diastereoselectivity (ca. 4:1 from ^1H NMR).

Scheme 3[a]



[a] Conditions: (a) TMS−C≡C−Li, THF, −78 °C. (b) PDC, 3 Å MS, CH_2Cl_2, rt. (c) $NaBH_4$, $CeCl_3(H_2O)_7$, MeOH, 0 °C. (d) Ac_2O, DMAP, Py; H_2O, rt. (e) $RuCl_3(H_2O)_n$, $NaIO_4$, CCl_4, H_2O, CH_3CN, rt. (f) PPTS, CH_2Cl_2, rt. (g) K_2CO_3, MeOH, 0 °C. (h) $HIO_4(H_2O)_2$, AcOMe, rt; MeOH, reflux.

secondary alcohol (C_9−OH) was proved to be the opposite of that of tetrodotoxin.[17] Consequently, the configuration was inverted by oxidation with PDC followed by the Luche reduction[18] to give an unstable propargyl alcohol,[19] which was subjected to acetylation to afford diacetate **14**[20] in 71% yield.[21] The stereochemical outcomes of these highly stereoselective reactions can be uniformly explained by invoking the similar chelation intermediates **a** and **b**, as depicted in Figure 2. In this model, the carbonyl oxygen, amide nitrogen,

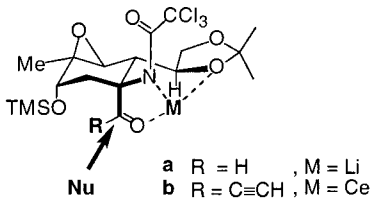
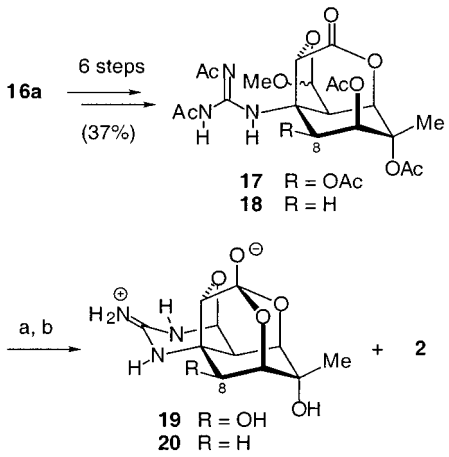


Figure 2.

and one oxygen of the acetal functionality were ligated by the metals (Li and Ce). The steric hindrance of the acetonide led nucleophiles such as acetylide and hydride to attack from the less sterically hindered front side. The alkynyl group of **14** was oxidatively cleaved with RuO_4 to give carboxylic acid.[22] Since spontaneous lactonization through epoxide opening was very sluggish under the conditions, the carboxylic acid was treated with acid to afford the lactone **15** in good yield. At this stage, the configuration of the C-9 position was confirmed by observing W-shaped long-range coupling (J = 1 Hz) between H-9 and H-4a of **15**. Prior to installation of the guanidine functionality, the hydroxyl group at the C-9 position was protected as an intramolecular mixed acetal with the aldehyde at the C-4 position in three steps. Selective deacetylation at C-9, oxidative cleavage of acetonide,[23] and acetalization in refluxing methanol provided a 4:1 separable mixture of **16a** and **16b**.[24] Now these compounds were set for guanidinylation.

In the total synthesis of 11-deoxytetrodotoxin **2**,[8] a two-step deprotection of fully functionalized intermediate **17** furnished 11-deoxytetrodotoxin **2** and 4,9-anhydro-11-deoxytetrodotoxin **19**, as shown in Scheme 4. Therefore, we

Scheme 4[a]



[a] Conditions: (a) aq NH_3, H_2O, MeOH, rt. (b) TFA, H_2O, rt.

synthesized a similar intermediate **18** from the major isomer **16a** in a manner analogous to that described for the synthesis of **2** and **19**.[25,26] Surprisingly, deprotection of **18** did not give either 8,11-dideoxytetrodotoxin **3** or its anhydro derivative **20** under the same reaction conditions as those of 11-deoxytetrodotoxin **2**.[27] This unexpected result implied that

(17) Determination of the configuration of C-9 is included in Supporting Information.

(18) Luche, J. L.; Gamal, A. L. *J. Am. Chem. Soc*. **1979**, *101*, 5848.

(19) The TMS group of acetylene was removed when the crude ynone was dissolved in MeOH dried over 3 Å molecular sieves.

(20) We found that a small amount of water drove the in situ deprotection of TMS ether and subsequent acetylation to give **14** in one pot.

(21) A series of these reactions were performed without purification, because of the instability of the intermediates.

(22) Carisen, P. H.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.

(23) Xie, M.; Berges, D. A.; Robins, M. J. *J. Org. Chem.* **1996**, *61*, 5178.

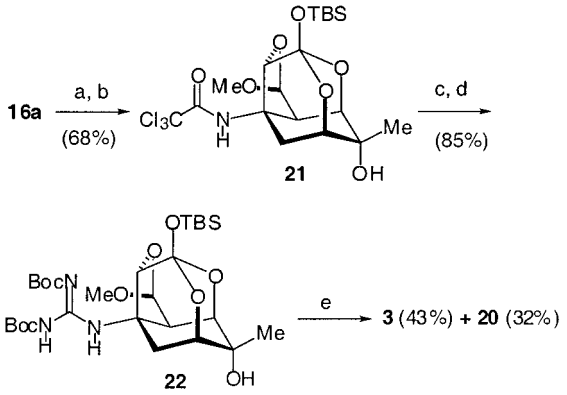
(24) Configurations of the C-4 position of **16a** and **16b** were established to be *S* and *R* by coupling constants between H-4 and H-4a (0 and 4 Hz, respectively). See ref 8.

(25) Details will be disclosed in a full account of this study.

(26) Nishikawa, T.; Ohyabu, N.; Yamamoto, N.; Isobe, M. *Tetrahedron* **1999**, *55*, 4325.

(27) Structures of the products have not been fully elucidated, because of the instability.

Scheme 5. Synthesis of 8,11-Dideoxytetrodotoxin[a]



[a] Conditions: (a) K_2CO_3, MeOH, rt. (b) TBS−OTf, Py, CH_3CN, rt. (c) DIBAL-H, CH_2Cl_2, −78 °C. (d) BocN═C(SMe)NHBoc, $HgCl_2$, Et_3N, DMF, rt. (e) TFA, MeOH, H_2O, rt.

the hydroxyl group at the C-8 position might contribute to the stabilization of the tetrodotoxin molecule, especially under basic conditions.

Considering the inapplicability of the deprotection procedure in this synthesis, we slightly modified the synthetic plan to protect the ortho ester function itself instead of protecting the δ-hydroxy lactone. Fortunately, upon treatment of **16a** with K_2CO_3 in MeOH at room temperature, acetate at the C-7 position was hydrolyzed to afford ortho ester, which was protected as silyl ether **21**.[28] The trichloroacetamide was reductively removed with DIBAL-H[29] to give an amine, which was subsequently guanidinylated with bis Boc-*S*-methylisothiourea[30] in the presence of $HgCl_2$[31] to afford **22** in high overall yield. Toward the goal, **22** was treated with aqueous TFA to furnish 8,11-dideoxytetrodotoxin **3** and 4,9-anhydro-8,11-dideoxytetrodotoxin **20** in 43 and 32% yields, respectively.[32] The structures of these products were confirmed by full assignment of ^1H and ^{13}C NMR spectra by extensive two-dimensional NMR experiments.

In summary, we have accomplished the synthesis of 8,11-dideoxytetrodotoxin in a highly stereoselective manner. This study provides a novel and efficient route for the synthesis of tetrodotoxin analogues through protection of the ortho ester and subsequent guanidine installation. Total synthesis of tetrodotoxin **1** along this line is currently under investigation.

Acknowledgment. This work was financially supported by JSPS-RFTF, a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, a Pfizer Award in Synthetic Organic Chemistry, and the Mitsubishi Chemical Corporation Fund.

Supporting Information Available: Proof of the configuration of the C-9 position of **13** and characterization data of all new compounds, including copies of the ^1H and ^{13}C NMR spectra of **3** and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) Heathcock, C. H.; Young, S. D.; Hagen, J. P.; Pilli, R.; Badertscher, U. *J. Org. Chem.* **1985**, *50*, 2095.

(29) Oishi, T.; Ando, K.; Inomoya, K.; Sato, H.; Iida, M.; Chida, N. *Org. Lett.* **2002**, *4*, 151.

(30) Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1987**, *52*, 1700.

(31) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.

(32) Due to the interconvertible nature of the tetrodotoxin analogue, 4,9-anhydro-8,11-dideoxytetrodotoxin **20** was equilibrated in 1% TFA-*d*/D_2O at room temperature for one week to reach a mixture of **3** and **20** in a 7:1 ratio (from ^1H NMR).