Total Synthesis of Tetrodotoxin

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Abstract: A total synthesis of tetrodotoxin was accomplished. A Diels-Alder reaction between a known enone and a siloxy diene gave a tricyclic product, the steric bias of which could be used to construct the remaining stereogenic centers. A nitrogen atom was introduced either via a four-step sequence involving a Curtius rearrangement, or a three-step sequence featuring a newly developed transformation of a terminal alkyne into a nitrile. Introduction of the guanidine moiety followed by the formation of the heterocyclic system via cascade reactions led to the synthesis of tetrodotoxin.

Tetrodotoxin is one of the most well studied and culturally revered natural products.[1] It is the toxin originally found in puffer fish,[2],[3] and is known to block voltage-gated sodium channels.[4] This biological activity has prompted the development of tetrodotoxin as a new class of analgesics, and clinical trials of tetrodotoxin are currently underway. The unique structure of tetrodotoxin, composed of a tetracyclic skeleton with a tricyclic orthoester (a dioxaadamantane core) and a cyclic guanidine moiety, is another attractive feature of the molecule (Figure 1).[5] The skeleton is highly oxidized and is substituted with five hydroxy groups. In addition, a variety of natural congeners have been isolated. Based on these structural relationships, biosynthetic pathways of tetrodotoxin have been proposed.[6] The structural complexity has made tetrodotoxin a fascinating synthetic target. Although several total syntheses and many synthetic studies of tetrodotoxin have been reported to date,[7] it deserves further synthetic efforts even today since biological and chemical behaviors of the densely functionalized molecules have not been fully controlled. Since our total synthesis of tetrodotoxin was reported in 2017,[7k] we have been pursuing investigations toward this attractive molecule with quite different synthetic strategies. Herein we disclose our novel total synthesis of tetrodotoxin.

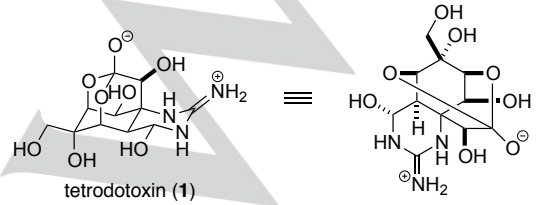
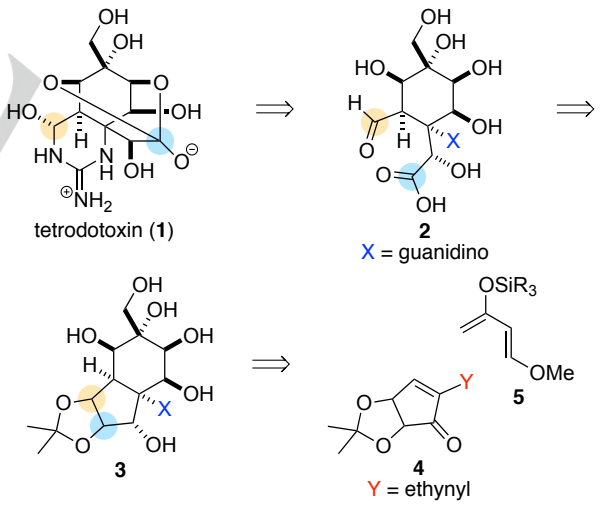


Figure 1. Structure of Tetrodotoxin.

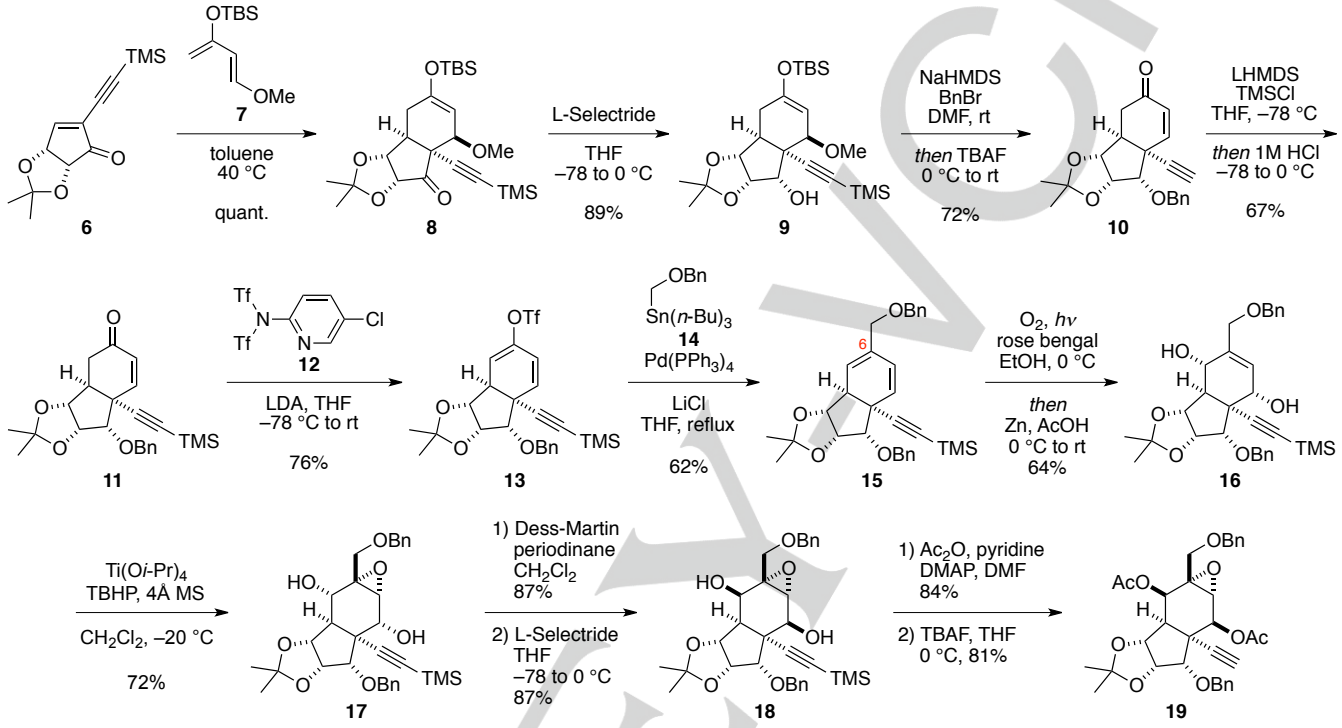
Our retrosynthetic analysis is shown in Scheme 1. The hemiaminal and orthoester moieties could be derived from aldehyde and carboxylic acid moieties, respectively. Connection of these two carbonyl groups would lead to *cis*-fused bicyclic compound **3**, in which a 1,2-diol moiety is installed for the oxidative cleavage. The hydroxy groups on the cyclohexane ring could be stereoselectively introduced using the steric bias of the *cis*-fused bicyclic skeleton, which could in turn be constructed via a Diels-Alder reaction, leading to cyclopentenone **4** and diene **5** as precursors. The functional group Y on the cyclopentenone ring should be an equivalent to the guanidino group. In addition, Y should contribute positively to the Diels-Alder reaction. To fulfill these requirements, we chose an ethynyl group as the functional group Y.[8]



Scheme 1. Retrosynthetic Analysis.

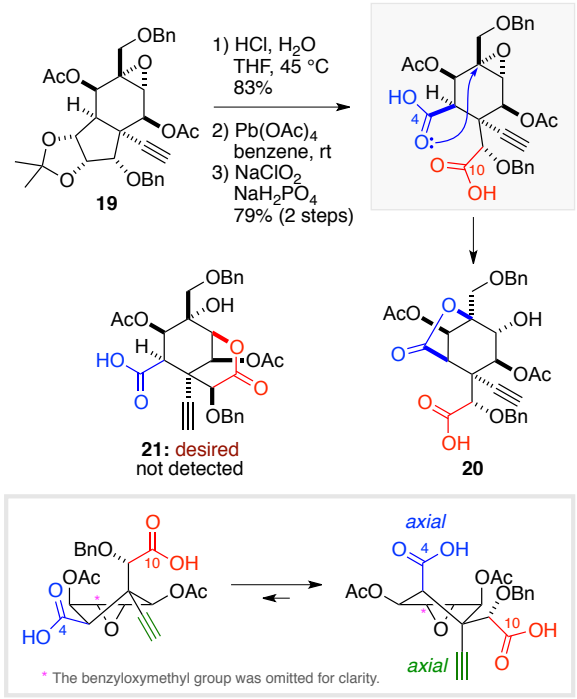
The synthesis commenced with preparation of dienophlile **6** from α-methyl-D-mannoside according to the literatures.[9] The Diels-Alder reaction of **6** with siloxydiene **7** proceeded smoothly in toluene at 40 °C, giving the adduct **8** as the sole isomer in a quantitative yield (Scheme 2). The ketone moiety in **8** was stereoselectively reduced with L-Selectride to furnish secondary alcohol **9**, which was protected as its benzyl ether. Treatment with TBAF induced cleavage of the TBS group, liberating the cyclohexenone moiety with concomitant removal of the TMS group on the alkyne moiety. After reinstallation of a TMS group

at the terminal alkyne, enone **11** was converted into triflate **13** by treatment with LDA and Comins reagent (**12**).[10] Palladium-catalyzed cross coupling reaction of triflate **13** with benzyloxymethylstannane **14** installed a hydroxymethyl moiety at C6.[11] The Diels-Alder reaction with singlet oxygen occurred from the convex face of the *cis*-fused bicyclic skeleton in **15**, and the resulting endoperoxide was reduced with zinc to afford diol **16**. Epoxidation directed by the hydroxy groups proceeded stereoselectively to give epoxide **17**. A two-step oxidation-reduction sequence inverted the configuration of the hydroxy groups to furnish **18**, which has the correct stereochemistry of tetrodotoxin. Protection of the hydroxy groups with acetyl groups, and subsequent removal of the TMS group with TBAF gave **19**.



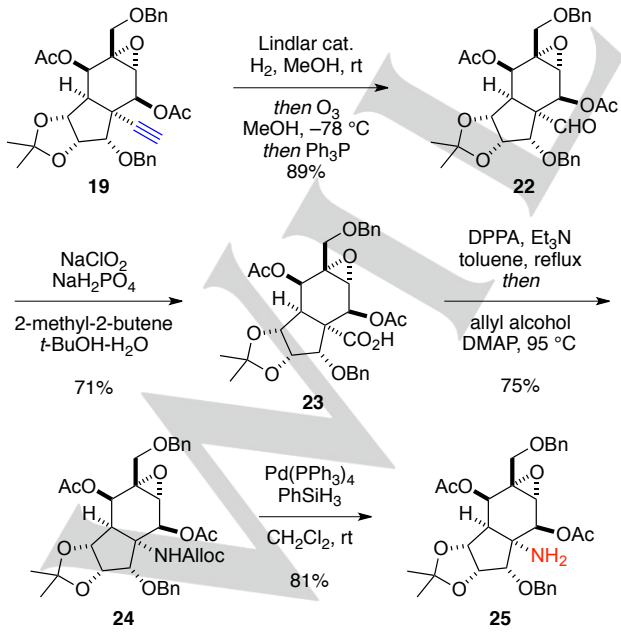
Scheme 2. Construction of the Cyclohexane Ring and Stereoselective Installation of the Hydroxy Groups.

We next focused on the formation of the dioxaadamantane skeleton. After acidic hydrolysis of the acetonide in **19**, the resulting 1,2-diol was oxidatively cleaved with lead tetraacetate (Scheme 3). Attempted oxidation of the resulting dialdehyde with sodium chlorite,[12] however, induced epoxide opening by the C4 carboxyl group (shown in blue) instead of C10 carboxyl group (shown in red), leading to the exclusive formation of the undesired lactone **20**.[13] This might be attributed to the sterically less demanding ethynyl group, which was inclined to assume the axial conformation in the cyclohexane ring. As a consequence, the C4 carboxyl group also took the axial conformation and attacked the epoxide.



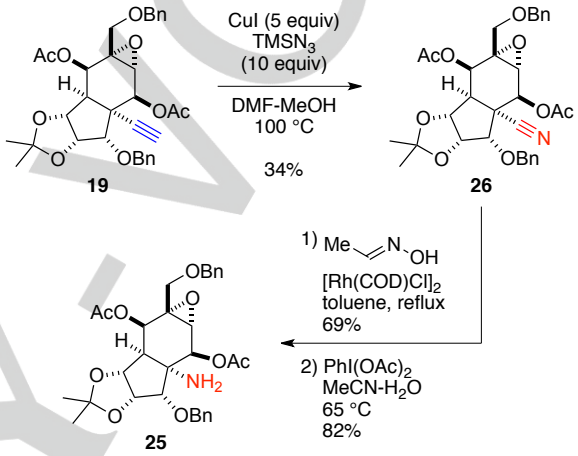
Scheme 3. Attempted Formation of the Lactone.

To change the conformation of the cyclohexane ring, we decided to convert the ethynyl group into the sterically more demanding guanidino group. For this transformation, the ethynyl group was converted into an amino group by a four-step sequence (Scheme 4). Partial reduction of the ethynyl group followed by ozonolysis gave aldehyde **22**, which was subjected to oxidation with sodium chlorite.[12] The resulting carboxylic acid **23** was converted into amine **25** via a Curtius rearrangement with DPPA[14] and allyl alcohol and subsequent cleavage of the Alloc group.



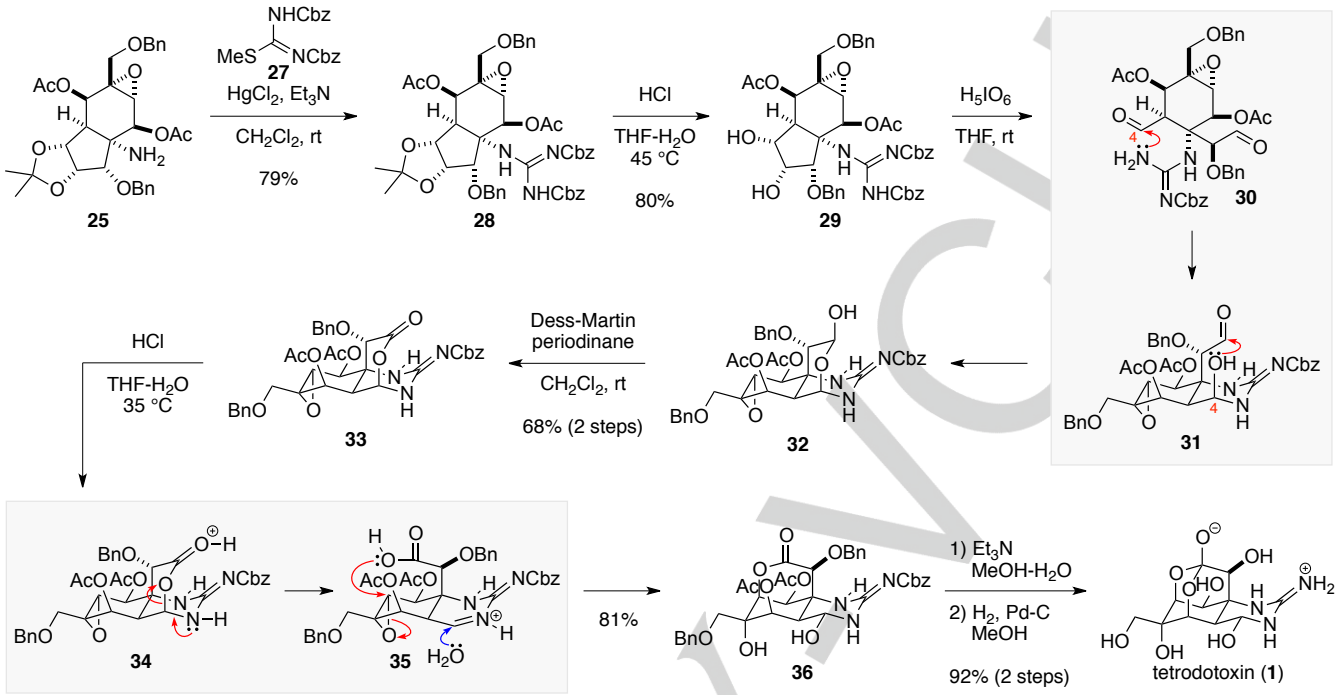
Scheme 4. Conversion of Alkyne into Amine via Curtius Rearrangement.

While investigating the other transformations of alkyne **19**, we found an unusual reaction in which the ethynyl group was converted into a cyano group (Scheme 5). Thus, treatment of **19** with CuI and $TMSN_3$ in methanol and DMF at 100 °C produced nitrile **26** in 34% yield.[15],[16] The nitrile could be hydrated according to Lee's protocol with a slight modification.[17] The resulting carboxamide was subjected to the modified Hofmann rearrangement to give amine **25**.[18] Although the yield left room for improvement, this protocol produced the desired amine **25** in three steps from alkyne **19**.



Scheme 5. Conversion of Alkyne into Nitrile.

The amine **25** was then converted to bis-Cbz protected guanidine **28** and the acetonide of which was subjected to acidic hydrolysis to furnish diol **29** (Scheme 6).[19] Oxidative cleavage of the 1,2-diol with orthoperiodic acid proceeded smoothly at room temperature. Under these conditions, the two formyl groups and the guanidino group in **30** cooperatively formed a tricyclic lactol. The guanidino group in the equatorial position attacked the more-accessible C4 formyl group to form hemiaminal **31**, the hydroxy group of which then attacked the other formyl group. The cyclic hemiacetal **32** could be oxidized into lactone **33** with Dess-Martin periodinane.[20]
Attempted cleavage of the lactone moiety in **33** with ammonia[21] caused decomposition of the substrate, which contained base-labile functional groups such as an epoxide and acetates. To our delight, treatment of **33** with hydrochloric acid in THF at 35 °C successfully cleaved the lactone moiety. The liberated carboxyl group attacked the epoxide at the less hindered position to afford the desired tricyclic lactone **36**. After removal of the acetyl groups under basic conditions, hydrogenolysis of the Cbz and the two benzyl groups gave tetrodotoxin (**1**).



Scheme 6. Completion of the Synthesis.

In conclusion, we have achieved the total synthesis of tetrodotoxin. A stereoselective Diels-Alder reaction produced a tricyclic compound. The steric bias of the resulting ring system could be used to construct other stereogenic centers. The alkyne moiety was converted into an amino group via a four-step sequence including Curtius rearrangement, or via a three-step sequence by means of a newly developed transformation of alkyne into nitrile mediated by CuI and $TMSN_3$. After installation of an amidino group onto the amine, the cyclic guanidine moiety was formed by oxidative cleavage of a 1,2-diol moiety, where two aldehyde units were efficiently differentiated by a nucleophilic attack of the guanidine moiety to give a cyclic hemiacetal. Oxidation into a lactone followed by an acidic treatment liberated the carboxyl group, which intramolecularly attacked the epoxide. Finally, deprotection gave tetrodotoxin.

Acknowledgements

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Keywords: natural products • Diels-Alder reaction • rearrangement • nitrile • guanidine

[1] E. G. Moczydlowski, *Toxicon* **2013**, *63*, 165-183.
[2] Y. Tahara, *J. Pharm. Soc. Jpn.* **1909**, 587-625.
[3] a) H. S. Mosher, F. A. Fuhrman, H. D. Buchwald, H. G. Fischer, *Science* **1964**, *144*, 1100-1110; b) T. Noguchi, Y. Hashimoto, *Toxicon* **1973**, *11*, 305-307; c) Y. H. Kim, G. B. Brown, F. A. Mosher, *Science* **1975**, *189*, 151-152; d) D. D. Sheumack, M. E. Howden, I. Spence, R. J. Quinn, *Science* **1978**, *199*, 188-189; e) T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo, Y. Kotaki, *Agric. Biol. Chem.* **1986**, *50*, 793-795; f) T. Noguchi, O. Arakawa, *Mar. Drugs* **2008**, *6*, 220-242; g) C. T. Hanifin, *Mar. Drugs* **2010**, *8*, 577-593.
[4] a) T. Narahashi, T. Deguchi, N. Urakawa, Y. Ohkubo, *Am. J. Physiol.* **1960**, *198*, 934-938; b) T. Narahashi, J. W. Moore, W. R. Scott, *J. Gen. Physiol.* **1964**, *47*, 965; c) H. Terlau, S. H. Heinemann, W. Stühmer, M. Pusch, F. Conti, K. Imoto, S. Numa, *FEBS Lett.* **1991**, *293*, 93-96; d) W. A. Catterall, *Neuron* **2000**, *26*, 13-25; e) C. Rosker, B. Lohberger, D. Hofer, B. Steinecker, S. Quasthoff, W. Schreibmayer, *Am. J. Physiol. Cell Physiol.* **2007**, *293*, C783-C789; f) T. Tsukamoto, Y. Chiba, M. Wakamori, T. Yamada, S. Tsunogae, Y. Cho, R. Sakakibara, T. Imazu, S. Tokoro, Y. Satake, M. Adachi, T. Nishikawa, M. Yotsu-Yamashita, K. Konoki, *Br. J. Pharmacol.* **2017**, *174*, 3881-3892; g) H. Shen, Z. Li, Y. Jiang, X. Pan, J. Wu, B. Cristofori-Armstrong, J. J. Smith, Y. K. Y. Chin, J. Lei, Q. Zhou, G. F. King, N. Yan, *Science* **2018**, *362*, eaau2596; h) H. Shen, D. Liu, K. Wu, J. Lei, N. Yan, *Science* **2019**, *363*, 1303-1308; i) L. Xu, X. Ding, T. Wang, S. Mou, H. Sun, T. Hou, *Drug Discovery Today* **2019**, *24*, 1389-1397.
[5] a) K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, K. Sakai, C. Tamura, O. Amakasu, *Chem. Pharm. Bull.* **1964**, *12*, 1357-1374; b) R. B. Woodward, *Pure Appl. Chem.* **1964**, *9*, 49-74; c) T. Goto, Y. Kishi, S. Takahashi, Y. Hirata, *Tetrahedron* **1965**, *21*, 2059-2088.
[6] a) T. Yasumoto, M. Yotsu, M. Murata, H. Naoki, *J. Am. Chem. Soc.* **1988**, *110*, 2344-2345; b) T. Yasumoto, M. Yotsu-Yamashita, *J. Toxicol. Toxin Rev.* **1996**, *15*, 81-90; c) M. Yotsu-Yamashita, *J. Toxicol. Toxin Rev.* **2001**, *20*, 51-66; d) J.-H. Jang, M. Yotsu-Yamashita, *Toxicon* **2007**, *50*, 947-951; e) M. Yotsu-Yamashita, Y. Abe, Y. Kudo, R. Ritson-Williams, J. V. Paul, K. Konoki, Y. Cho, M. Adachi, T. Imazu, T.

Nishikawa, M. Isobe, *Mar. Drugs* **2013**, *11*, 2799-2813; f) Y. Kudo, Y. Yamashita, D. Mebs, Y. Cho, K. Konoki, T. Yasumoto, M. Yotsu-Yamashita, *Angew. Chem. Int. Ed.* **2014**, *53*, 14546-14549; *Angew. Chem.* **2014**, *126*, 14774-14777; g) Y. Kudo, T. Yasumoto, D. Mebs, Y. Cho, K. Konoki, M. Yotsu-Yamashita, *Angew. Chem. Int. Ed.* **2016**, *55*, 8728-8731; *Angew. Chem.* **2014**, *128*, 8870-8873; h) N. Ueyama, K. Sugimoto, Y. Kudo, K.-I. Onodera, Y. Cho, K. Konoki, T. Nishikawa, M. Yotsu-Yamashita, *Chem. Eur. J.* **2018**, *24*, 7250-7258; i) M. Adachi, T. Miyasaka, Y. Kudo, K. Sugimoto, M. Yotsu-Yamashita, T. Nishikawa, *Org. Lett.* **2019**, *21*, 780-784; j) Y. Kudo, M. Yotsu-Yamashita, *J. Nat. Prod.* **2019**, *82*, 1656-1663.

[7] a) Y. Kishi, M. Aratani, T. Fukuyama, Nakatsub.F, T. Goto, S. Inoue, H. Tanino, S. Sugiura, H. Kakoi, *J. Am. Chem. Soc.* **1972**, *94*, 9217-9219; b) Y. Kishi, T. Fukuyama, M. Aratani, F. Nakatsubo, T. Goto, S. Inoue, H. Tanino, S. Sugiura, H. Kakoi, *J. Am. Chem. Soc.* **1972**, *94*, 9219-9221; c) N. Ohyabu, T. Nishikawa, M. Isobe, *J. Am. Chem. Soc.* **2003**, *125*, 8798-8805; d) A. Hinman, J. Du Bois, *J. Am. Chem. Soc.* **2003**, *125*, 11510-11511; e) T. Nishikawa, D. Urabe, M. Isobe, *Angew. Chem. Int. Ed.* **2004**, *43*, 4782-4785; *Angew. Chem. Int. Ed.* **2004**, *116*, 4886-4889; f) K.-I. Sato, S. Akai, N. Sugita, T. Ohsawa, T. Kogure, H. Shoji, J. Yoshimura, *J. Org. Chem.* **2005**, *70*, 7496-7504; g) K.-I. Sato, S. Akai, H. Shoji, N. Sugita, S. Yoshida, Y. Nagai, K. Suzuki, Y. Nakamura, Y. Kajihara, M. Funabashi, J. Yoshimura, *J. Org. Chem.* **2008**, *73*, 1234-1242; h) F. Cagide-Fagín, R. Alonso, *Eur. J. Org. Chem.* **2010**, *2010*, 6741-6747; i) S. Xu, M. A. Ciufolini, *Org. Lett.* **2015**, *17*, 2424-2427; j) S. Akai, H. Seki, N. Sugita, T. Kogure, N. Nishizawa, K. Suzuki, Y. Nakamura, Y. Kajihara, J. Yoshimura, K.-I. Sato, *Bull. Chem. Soc. Jpn.* **2010**, *83*, 279-287; k) T. Maehara, K. Motoyama, T. Toma, S. Yokoshima, T. Fukuyama, *Angew. Chem. Int. Ed.* **2017**, *56*, 1549-1552; *Angew. Chem.* **2017**, *129*, 1571-1574; l) D. Baidilov, L. Rycek, J. F. Trant, J. Froese, B. Murphy, T. Hudlicky, *Angew. Chem. Int. Ed.* **2018**, *57*, 10994-10998; *Angew. Chem.* **2018**, *130*, 11160-11164.

[8] S.-J. Min, G. O. Jones, K. N. Houk, S. J. Danishefsky, *J. Am. Chem. Soc.* **2007**, *129*, 10078-10079.

[9] a) M. W. Miller, C. R. Johnson, *J. Org. Chem.* **1997**, *62*, 1582-1583; b) H. Kumamoto, K. Deguchi, T. Wagata, Y. Furuya, Y. Odanaka, Y. Kitade, H. Tanaka, *Tetrahedron* **2009**, *65*, 8007-8013.

[10] D. L. Comins, A. Dehghani, *Tetrahedron Lett.* **1992**, *33*, 6299-6302.

[11] W. C. Still, *J. Am. Chem. Soc.* **1978**, *100*, 1481-1487.

[12] B. O. Lindgren, T. Nilsson, *Acta Chem. Scand.* **1973**, *27*, 888– 890; b) G. A. Kraus, M. J. Taschner, *J. Org. Chem.* **1980**, *45*, 1175-1176; c) G. A. Kraus, B. Roth, *J. Org. Chem.* **1980**, *45*, 4825-4830; d) B. S. Bal, W. E. Childers, H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091-2096.

[13] The structure of **20** was confirmed after conversion into the corresponding methyl ester. For details, see Supporting Information.

[14] a) T. Shioiri, K. Ninomiya, S. Yamada, *J. Am. Chem. Soc.* **1972**, *94*, 6203-6205; b) T. Shioiri, S. Yamada, *J. Synth. Org. Chem. Jpn.* **1973**, *31*, 666-674.

[15] For related reactions employing CuI and TMSN3 to produce *N*-unsubstituted 1,2,3-triazoles, see: T. Jin, S. Kamijo, Y. Yamamoto, *Eur. J. Org. Chem.* **2004**, *2004*, 3789-3791.

[16] For related reactions which convert alkynes into nitriles under other conditions, see: a) N. Okamoto, M. Ishikura, R. Yanada, *Org. Lett.* **2013**, *15*, 2571-2573; b) T. Shen, T. Wang, C. Qin, N. Jiao, *Angew. Chem. Int. Ed.* **2013**, *52*, 6677-6680; c) U. Dutta, D. W. Lupton, D. Maiti, *Org. Lett.* **2016**, *18*, 860-863; d) S. Xu, T. Cai, Z. Yun, *Synlett* **2016**, *27*, 221-224.

[17] J. Lee, M. Kim, S. Chang, H.-Y. Lee, *Org. Lett.* **2009**, *11*, 5598-5601.

[18] R. M. Moriarty, C. J. Chany, R. K. Vaid, O. Prakash, S. M. Tuladhar, *J. Org. Chem.* **1993**, *58*, 2478-2482.

[19] K. S. Kim, L. Qian, *Tetrahedron Lett.* **1993**, *34*, 7677-7680.

[20] D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155-4156.

[21] A. Ogura, K. Yamada, S. Yokoshima, T. Fukuyama, *Org. Lett.* **2012**, *14*, 1632-1635.

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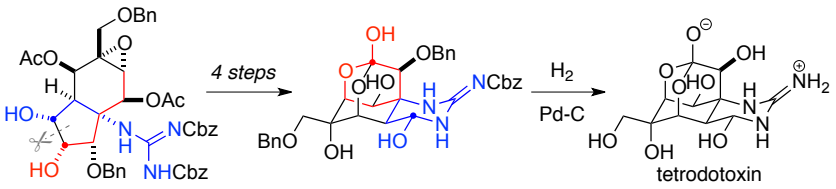

A total synthesis of tetrodotoxin was accomplished. Construction of the cyclohexane core via a Diels-Alder reaction was followed by stereoselective installation of hydroxy groups. A newly developed transformation of a terminal alkyne into a nitrile enabled introduction of an amino group. Oxidative cleavage of a 1,2-diol formed two aldehyde units, which were differentiated by nucleophilic attack of a guanidine moiety, leading to tetrodotoxin.