Natural Product Synthesis

An Efficient Total Synthesis of Optically Active Tetrodotoxin**

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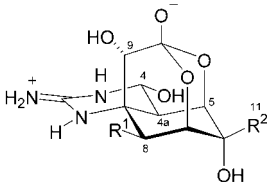
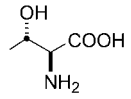
Tetrodotoxin (TTX, **1**), a toxic principle of puffer-fish poison, is one of the most famous natural products because of its novel structure coupled with its potent biological activity.[1] The structure was revealed by Hirata, Goto, and co-workers,



1 R^1 = OH R^2 = CH_2OH
2 R^1 = OH R^2 = Me
3 R^1 = H R^2 = Me
4 R^1 = OH R^2 =



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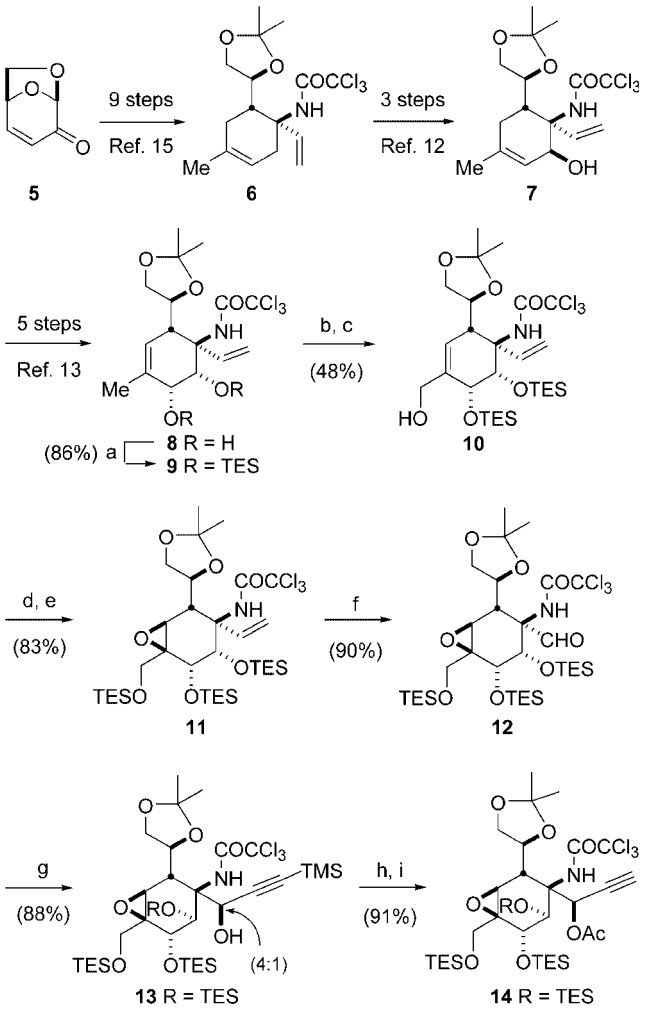
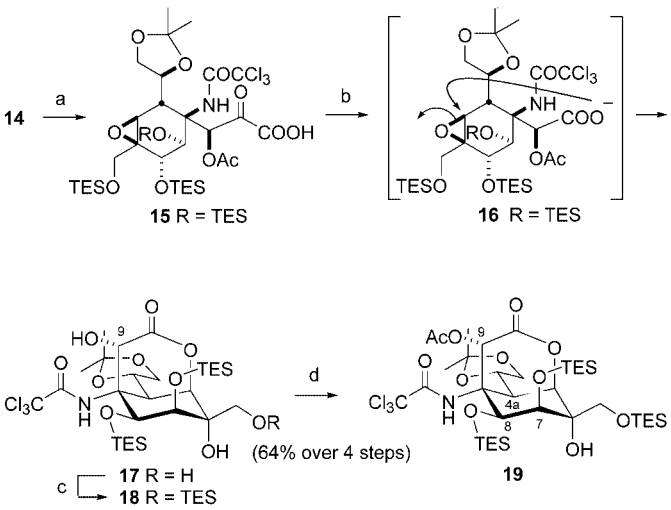
Tsuda et al., and Woodward in the 1960s[2] to be an unprecedented multifunctional structure that includes a polyhydroxylated dioxaadamantane unit with an ortho ester and a cyclic guanidine unit with a hemiaminal. The discovery that this toxin acts as a specific blocker of voltage-dependent sodium channels has led to its widespread use as a biochemical tool in neurophysiology.[3] The highly functionalized cage structure and unusual chemical properties of (−)-tetrodotoxin (**1**) had hindered efforts towards its synthesis[4] until our recent total synthesis,[5] although Kishi and co-workers had already reported the total synthesis of racemic tetrodotoxin in 1972.[6] Another total synthesis of **1** was reported by Hinman and Du Bois in 2003.[7] Over the last few decades, a variety of analogues of tetrodotoxin, such as 11-deoxytetrodotoxin (**2**), 5,6,11-trideoxytetrodotoxin, and chiriquitoxin (**4**), have been isolated not only from puffer fish, but from many other organisms living in oceanic and fresh water, as well as from terrestrial animals.[8] These new findings have raised several interesting issues, such as questions about the biosynthesis of tetrodotoxin and the organisms that produce it,[9] as well as about the resistance mechanism and the actual biological function of tetrodotoxin in puffer fish.[10,11] During the course of our synthetic studies on tetrodotoxin with the aim of analyzing such problems, we have reported the total synthesis of several naturally occurring and nonnatural tetrodotoxin analogues, such as 5,11-dideoxytetrodotoxin,[12] 11-deoxytetrodotoxin (**2**),[13] and 8,11-dideoxytetrodotoxin (**3**),[14] from a common synthetic intermediate **6** (Scheme 1).[15] We describe herein an efficient total synthesis of (−)-tetrodotoxin (**1**) on the basis of an alternative strategy to that used for the first total synthesis of **1** in our laboratory.[5]

The synthesis commenced with the hydroxylation of the vinylic methyl group of an intermediate **8** in our synthesis of 11-deoxytetrodotoxin.[13] This intermediate was prepared in 17 steps from the chiral starting material levoglucosenone (**5**) through the common intermediate **6**[15] and an intermediate **7** for 5,11-dideoxytetrodotoxin[12] (Scheme 1). Following protection of the diol as the bis(triethylsilyl) ether **9**, the allylic oxidation of **9** was carried out with selenium dioxide and pyridine *N*-oxide (PNO)[16] to give the corresponding unsaturated aldehyde, which was reduced under the Luche conditions to the allylic alcohol **10** in moderate overall yield. The protection of the resulting primary alcohol with a TES group was followed by treatment with *m*-chloroperbenzoic acid (MCPBA) to afford a single epoxide **11** in high yield. In analogy with the previous syntheses of tetrodotoxin analogues,[12–14] the vinyl group was cleaved by ozonolysis followed by in situ reduction with Et_3N to yield the aldehyde **12**, which was subjected to the stereoselective addition of acetylide as a carboxylic acid equivalent. In this case, the alkynyl magnesium bromide prepared from (trimethylsilyl)-acetylene and EtMgBr in THF was found to be the best reagent, and these conditions led to a 4:1 mixture of **13** from



Scheme 1. Reagents and conditions: a) TESOTf, pyridine, CH_3CN, room temperature; b) SeO_2, PNO, dioxane, reflux; c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C; d) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; e) MCPBA, Na_2HPO_4, CH_2Cl_2, room temperature; f) O_3, CH_2Cl_2, −78 °C, then Et_3N; g) TMS−C≡CH, EtMgBr, THF, 0 °C; h) Ac_2O, DMAP, pyridine, room temperature; i) TBAF, THF, −10 °C. TBAF = tetrabutylammonium fluoride, DMAP = 4-dimethylaminopyridine, TES = triethylsilyl, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.



Scheme 2. Reagents and conditions: a) $KMnO_4$, $NaIO_4$, $NaHCO_3$, *t*BuOH, H_2O, 50 °C; b) H_2O_2 (30%), $NaHCO_3$, MeOH, room temperature; c) TESOTf, 2,6-lutidine, CH_2Cl_2, −40 °C; d) Ac_2O, pyridine, room temperature.

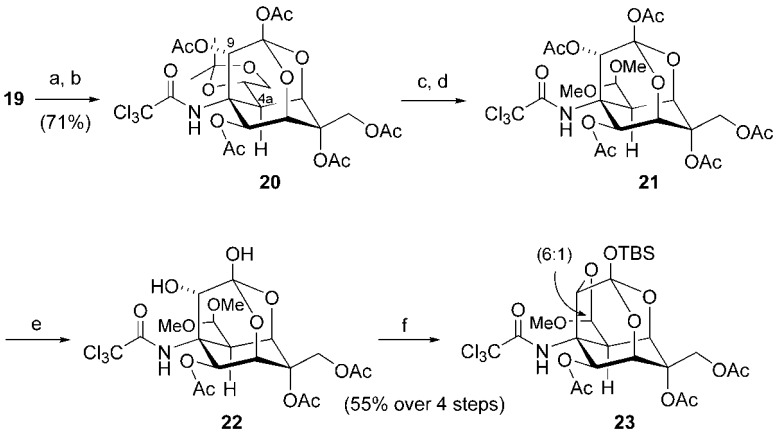
which the desired product was isolated in 70% yield.[17] The major product **13** was transformed by acetylation and removal of the TMS group into the propargyl acetate **14**, which was set for oxidative cleavage of the acetylenic moiety.

The attempted cleavage of the acetylenic moiety of **14** with ruthenium oxide, which had been employed in our previous syntheses of deoxytetrodotoxin analogues, failed to give the expected α-acetoxycarboxylic acid or lactone. The product obtained was instead found to be the α-keto acid **15**,[18] thus indicating steric congestion around the acetylenic moiety. Further experiments led us to find that $KMnO_4$ and $NaIO_4$ were the most efficient oxidants[19] of **14** to the α-keto acid **15**, which was cleaved with alkaline hydrogen peroxide to furnish a mixture of the desired lactone **18** and a partially desilylated product **17** (Scheme 2). The treatment of the crude reaction mixture with TESOTf and 2,6-lutidine effected resilylation to give **18**. Acetylation of the hydroxy group at C9 gave **19**, which contains a fully functionalized cyclohexane ring with the correct stereochemistry.

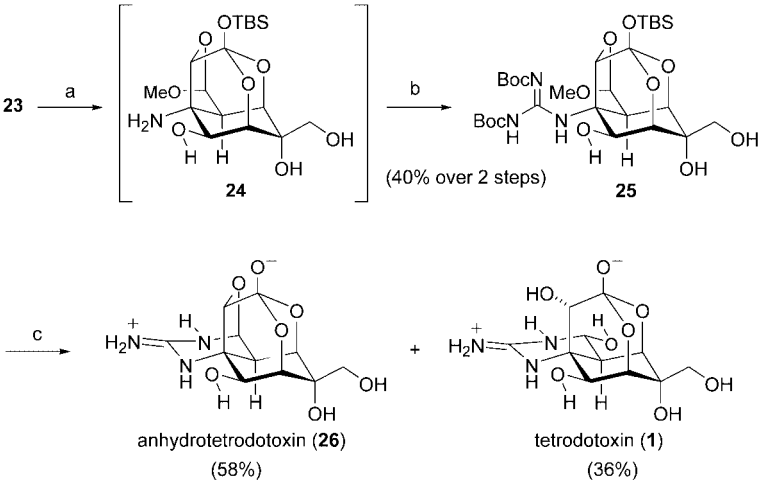
The remaining tasks in the total synthesis of tetrodotoxin were the cleavage of the 1,2-diol protected as an acetonide and the installation of the guanidine functionality. Major difficulties encountered at this stage were epimerization at C9 and the low nucleophilicity of the amino group obtained from the trichloroacetamide. After extensive investigations of protective-group strategies for hydroxy groups, we eventually found the following successful route via the key intermediate **23**, which contains an acetal and an ortho ester (Scheme 3). Thus, prior to cleavage of the acetonide with periodic acid, it was necessary to exchange the acid-sensitive TES protective groups for acetate groups. The removal of all TES groups in **19** with TBAF was followed by peracetylation to give pentaacetate **20**[20] in the form of an ortho ester.[21] Attempted desilylation of **18** (with a free 9-hydroxy group) under the same conditions resulted in exclusive deprotection of the 11-hydroxy group without epimerization, thus indicating that the presence of the acetate group on the 9-hydroxy group might be crucial for deprotection of TES groups at the 7- and 8-positions. The 1,2-acetonide was cleaved with periodic acid to give an aldehyde, which was immediately protected as the dimethylacetal **21**. Reductive cleavage of the trichloroacetamide with DIBAL-H[14,22] required protection of the ortho ester with a silyl group. Fortunately, we found that the treatment of **21** with aqueous ammonia in MeOH afforded **22** exclusively. Compound **22** was treated with an excess of TBSOTf to give a 6:1 inseparable mixture of the acetal **23**.[21,23]

All acyl protecting groups in **23** were removed with DIBAL-H at −40 °C for 8 h (Scheme 4).[24] The resulting amine **24** was then guanidinylated with bis(Boc-(*S*)-methylisothiourea) in the presence of mercuric chloride[25] to give **25**, a suitably protected precursor of tetrodotoxin. Exposure of **25** to aqueous TFA caused global deprotection and subsequent formation of the cyclic guanidine moiety to furnish tetrodotoxin (**1**) and 4,9-anhydrotetrodotoxin (**26**) in 36 and 58% yield, respectively, after purification by HPLC on an ion-exchange resin. The synthetic material **1** proved to be identical in all respects to natural tetrodotoxin.

In summary, the total synthesis of tetrodotoxin from **8** has been accomplished in a highly concise manner, which should enable us to supply a variety of tetrodotoxin derivatives that are not readily accessible from natural products for biochemical studies.



Scheme 3. Reagents and conditions: a) TBAF, CH_3CN, 0 °C; b) Ac_2O, pyridine, room temperature; c) HIO_4, AcOMe, room temperature; d) CSA, $HC(OMe)_3$, MeOH, room temperature; e) aqueous NH_3, MeOH, 0 °C; f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C. CSA = camphor sulfonic acid, TBS = *tert*-butyldimethylsilyl.



Scheme 4. Reagents and conditions: a) DIBAL-H, CH_2Cl_2, −40 °C; b) BocN=C(SMe)NHBoc, $HgCl_2$, Et_3N, DMF, room temperature; c) TFA, H_2O, room temperature. Boc = *tert*-butoxycarbonyl, DIBAL-H = diisobutylaluminum hydride, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid.

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[21] The structure of the ortho ester was confirmed by ^{13}C NMR spectroscopy; $\delta = 108.2$ ppm for compound **20**, $\delta = 108.5$ ppm for compound **23**.

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