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Total Synthesis of Chiriquitoxin, an Analogue of Tetrodotoxin Isolated from the Skin of a Dart Frog

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Abstract: The first total synthesis of chiriquitoxin, the most structurally complex analogue of tetrodotoxin isolated from a Costa Rican dart frog, has been accomplished from a newly designed intermediate for a variety of tetrodotoxin derivatives. The synthesis includes the third total synthesis of tetrodotoxin in this laboratory, and its intermediate was transformed into chiriquitoxin by a stereocontrolled aldol reaction with a D-camphor-derived lactone for installation of the unique side chain, and a new deprotection of methylthiomethyl (MTM) ether by using a Pummerer rearrangement.

In 1975, Mosher and co-workers reported isolation of chiriquitoxin, a potent new neurotoxin, along with tetrodotoxin (TTX, **1**) from the skin of a Costa Rican dart frog, *Atelopus chiriquensis*.[1–3] The molecular weight of this new compound was determined in 1976 by ^{252}Cf plasma desorption mass spectroscopy to be 393.[4] The NMR spectra were very similar to those of tetrodotoxin (**1**), a toxic principle of puffer fish intoxication;[5] however, the structure had not been elucidated until 15 years after the discovery. In 1990, Yasumoto, Yotsu–Yamashita, and co-workers successfully elucidated the structure of chiriquitoxin (CHTX, **2**) as shown in Figure 1 by extensive analysis of NMR spectra and derivatization reactions.[6] Chiriquitoxin is an analogue of tetrodotoxin, in which a glycine residue is connected to the C-11 position of tetrodotoxin.

Toxicity and inhibitory activity against the voltage-gated sodium channel of chiriquitoxin resembles those of tetrodotoxin.[7,8] In 1981, Kao and co-workers reported that chiriquitoxin is the first tetrodotoxin analogue shown to interfere with the outwardly rectifying potassium channel.[7b] These characteristic biological activities of **2** have attracted considerable interest in the field of neurophysiology as with tetrodotoxin and saxitoxin.[9] However, the scarcity of chiriquitoxin from natural sources has prevented further detailed investigations of the mechanisms of the ion-channel inhibitions. Thus, supply of **2** by chemical synthesis has been highly desired.

We have been synthesizing tetrodotoxin and its analogues for a long time.[10–18] In recent years, we have focused on developing a subtype selective blocker of a voltage-gated sodium channel based on tetrodotoxin and saxitoxin to analyze the biological functions. Concerning this issue, we have been interested in chiriquitoxin, because it is the sole example that possesses potassium-channel blocking activity among the tetrodotoxin family. Herein, we disclose the first asymmetric total synthesis of chiriquitoxin (**2**) from a newly designed intermediate for the syntheses of a wide variety of tetrodotoxin analogues.[19]

Our synthetic plan for chiriquitoxin is illustrated in Scheme 1.[20] Since the structural difference between tetrodotoxin and chiriquitoxin exists at the side chain at the C-11 position, we would synthesize chiriquitoxin by addition of a glycine

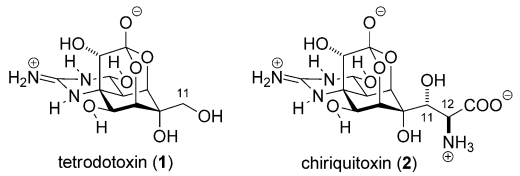
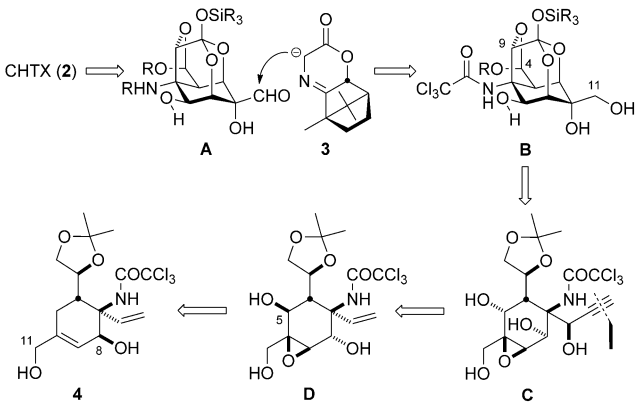


Figure 1. Structures of tetrodotoxin (**1**) and chiriquitoxin (**2**).



Scheme 1. Synthetic plan for chiriquitoxin (**2**) from a newly designed intermediate **4**.

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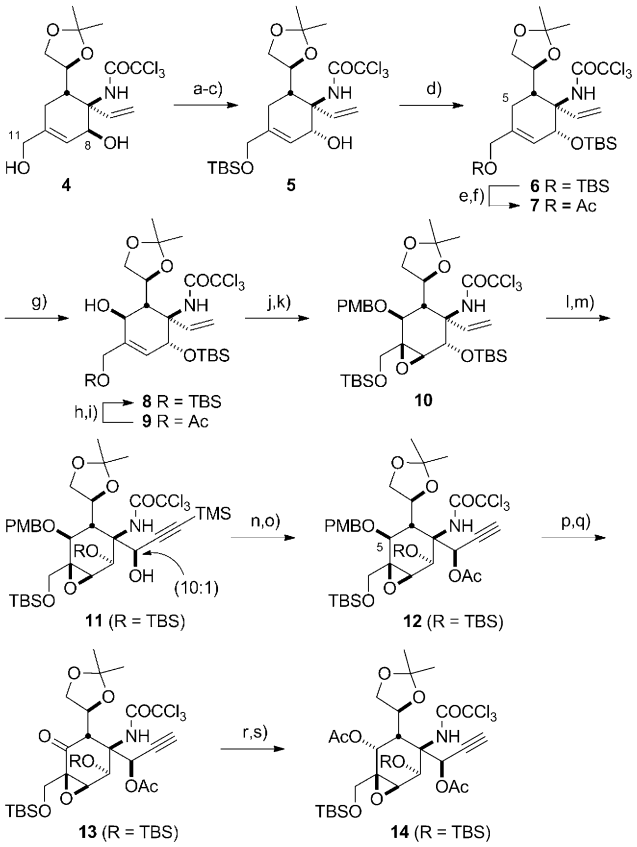
unit to the aldehyde at the C-11 position of an intermediate. The required aldehyde **A** could be obtained by oxidation of the corresponding hydroxy group at the C-11 position of the intermediate **B**, which would also be a promising intermediate for tetrodotoxin. In order to synthesize the β-hydroxy amino acid of chiriquitoxin stereoselectively, we selected a D-camphor-derived tricycloiminolactone **3** as a chiral glycine equivalent, because a highly *anti*-selective aldol reaction with aldehyde was reported by Xu and co-workers.[21] The intermediate **B** was envisaged to arise from **C** by oxidative cleavage of the acetylenic moiety to carboxylic acid and subsequent orthoester formation. According to the previous synthetic studies in this laboratory, the acetylene **C** would be synthesized from vinylepoxide **D** through ozonolysis of the vinyl group followed by stereoselective addition of acetylide as a carboxylic acid equivalent. The oxygen functionalities of **D** would be installed through hydroxylation at the C-5 position by allylic oxidation and subsequent epoxidation from compound **4**,[19] a new intermediate possessing two hydroxy groups at the C-8 and C-11 positions for tetrodotoxin and its analogues.

Synthesis began with inversion of the configuration of the hydroxy group at the C-8 position of the intermediate **4** (Scheme 2). Protection of the primary alcohol with a TBS group and oxidation with PCC gave enone, which was reduced with $LiAlH_4$ in ether as a solvent at approximately −110 °C, giving the desired allylic alcohol **5** in good yield with high stereoselectivity (d.r. > 20:1).[22] Protection of the resulting alcohol with a TBS group provided **6**. Allylic oxidation at the C-5 position was next investigated. Upon heating of the bis-TBS ether **6** with SeO_2 and pyridine *N*-oxide in 1,4-dioxane,[10b–c] the desired allylic alcohol **8** was obtained in 15% yield along with an α,β-unsaturated aldehyde in 80% yield. Further experiments revealed that the regioselectivity depended on the protective group of the hydroxy group at the C-11 position.[23] In the event, the acetate **7** was found to be the best substrate for the allylic oxidation under the conditions, providing the desired **9** in 52% yield with the unsaturated aldehyde in 22% yield. Then, **9** was transformed into bis-TBS ether **8** in two steps because the acetyl group was not compatible for the subsequent reactions.

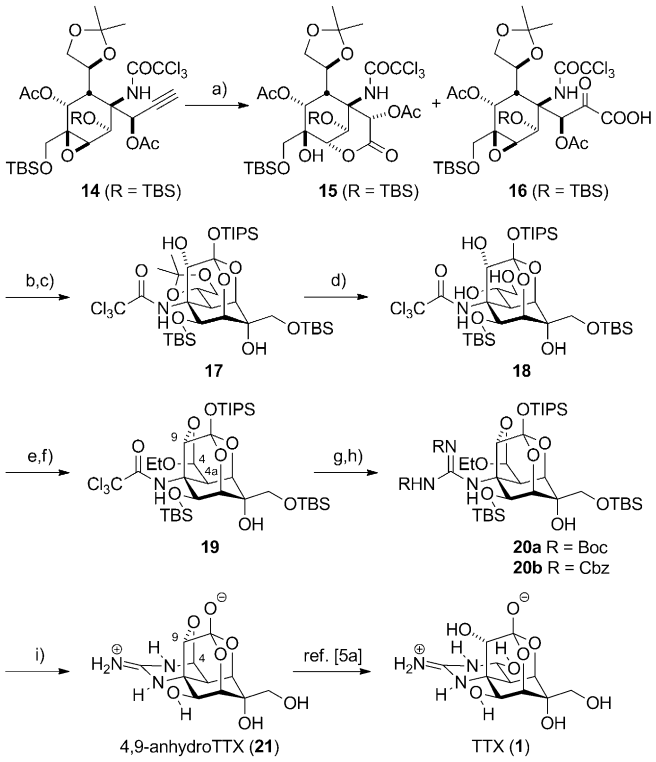
Epoxidation of **8** with MCPBA and subsequent protection of the secondary alcohol as a PMB ether gave **10**. The vinyl group was ozonolyzed to afford an aldehyde, which was subjected to addition of trimethylsilylacetylene as a carboxylic acid equivalent. The aldehyde reacted with the lithium acetylide in THF at −20 °C to give a 10:1 diastereomeric mixture, from which the desired product **11** was isolated in 80% yield in two steps from **10**.[24] Acetylation followed by selective desilylation of the TMS group afforded propargyl acetate **12**. At this stage, the configuration of the hydroxy group at the C-5 position of **12** was inverted by an oxidation–reduction sequence; deprotection of the PMB group with DDQ was followed by oxidation with PCC to give ketone **13**. Reduction with $NaBH_4$ provided a single alcohol, which was isolated as a diacetate **14** in 94% yield in two steps.

With multigram quantities of **14** in hand, oxidative cleavage of the acetylenic moiety and subsequent lactone formation

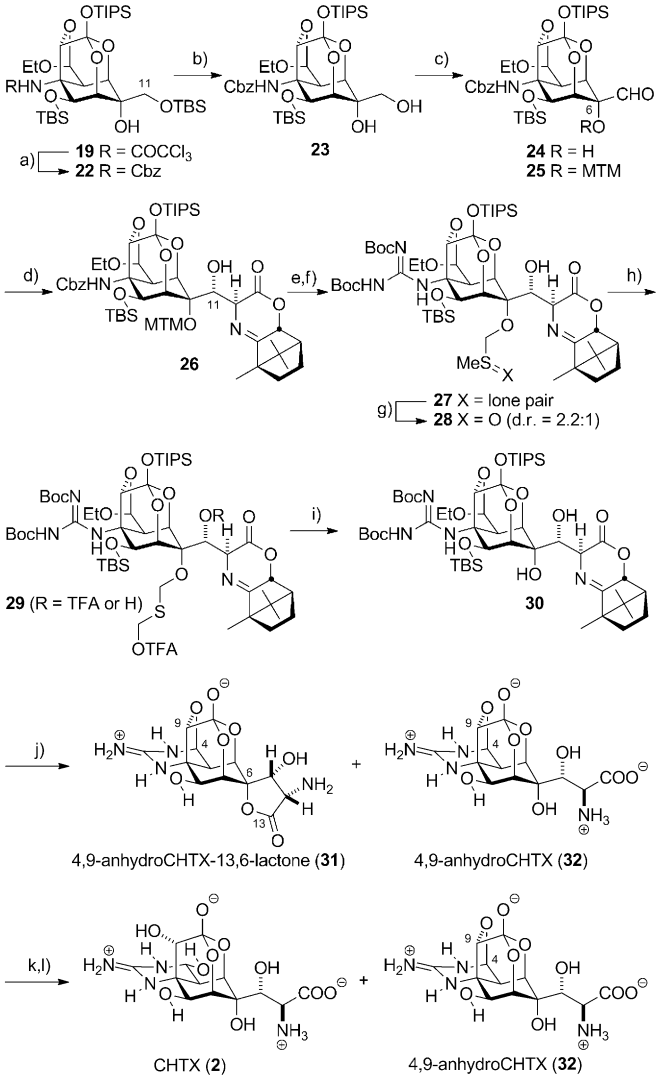


Scheme 2. Synthesis of diacetate **14**: a) TBSCl, imidazole, DMF, 0 °C, 15 min, 96%; b) PCC, 4 Å MS, CH_2Cl_2, RT, 12 h, 89%; c) $LiAlH_4$, Et_2O, ca. −110 °C, 0.5 h, 73%; d) TBSOTf, 2,6-lutidine, CH_3CN, −10 °C, 10 min, 95%; e) HF·Py, pyridine, THF, −20 °C, 5 h; f) Ac_2O, pyridine, RT, 1 h, 86% in 2 steps; g) SeO_2, PNO, 1,4-dioxane, 100 °C, 34 h, 52%; h) K_2CO_3, MeOH, RT, 30 min; i) TBSOTf, 2,6-lutidine, CH_2Cl_2, −20 °C, 0.5 h, 99% in 2 steps; j) MCPBA, $(CH_2Cl)_2$, RT, 19 h, 88%; k) PMBOC(=NH)CCl_3, TfOH, Et_2O, 0 °C, 0.5 h, 86%; l) O_3, CH_2Cl_2, −78 °C, 5 h, then Me_2S; m) trimethylsilylacetylene, *n*BuLi, THF, −20 °C, 0.5 h, 80% in 2 steps; n) Ac_2O, pyridine, RT, 2.5 h; o) TBAF, MeOH, THF, −20 °C, 15 min, 95% in 2 steps; p) DDQ, CH_2Cl_2, H_2O, RT, 4 h, 93%; q) PCC, MS-4 Å, CH_2Cl_2, RT, 12 h, 87%; r) $NaBH_4$, MeOH, −20 °C, 0.5 h; s) Ac_2O, pyridine, DMAP, RT, 11 h, 94% in 2 steps. TBS = *tert*-butyldimethylsilyl, PCC = pyridinium chlorochromate, MS = molecular sieves, Tf = trifluoromethanesulfonyl, Py = pyridine, THF = tetrahydrofuran, Ac = acetyl, PNO = pyridine *N*-oxide, MCPBA = 3-chloroperbenzoic acid, PMB = *p*-methoxybenzyl, TBAF = tetrabutylammonium fluoride, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = *N*,*N*-dimethyl-4-aminopyridine.

through epoxide opening was next investigated. Oxidation of **14** was best carried out by $RuCl_3{\cdot}nH_2O$ and Oxone[25] to give a mixture of the desired lactone **15** and α-ketocarboxylic acid **16** (Scheme 3).[26] Without purification, the mixture was treated with alkaline hydrogen peroxide to give the desired carboxylic acid, which underwent spontaneous opening of the epoxide to afford an equilibrium mixture of lactones and orthoester. The mixture was further treated with TIPSOTf in the presence of 2,6-lutidine to provide the desired orthoester **17** in 68% overall yield in three steps from **14**. To cleave the 1,2-glycol protected as an acetonide, deprotection of the acetonide was attempted. Although the selective deprotection in the presence of acid-sensitive protective groups such as siloxy groups was difficult, we fortunately found that **17** was treated with



Scheme 3. Synthesis of orthoester **19** and 4,9-anhydrotetrodotoxin (**21**): a) $RuCl_3{\cdot}nH_2O$, Oxone, $NaHCO_3$, CCl_4, CH_3CN, H_2O, RT, 20 h; b) H_2O_2 aq., $NaHCO_3$, MeOH, RT, 8 h; c) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 50 min, 68% in 3 steps; d) $SnCl_2{\cdot}2H_2O$, CH_2Cl_2, RT, 7 h, 66% (recovered starting material 15%); e) $NaIO_4$, MeOH, H_2O, RT, 2.5 h; f) PPTS, $CH(OEt)_3$, EtOH, RT, 12 h, 90% in 2 steps; g) DIBAL-H, CH_2Cl_2, −40 °C, 15 min; h) BocN=C(SMe)NHBoc, $HgCl_2$, Et_3N, DMF, RT, 20 min, 69% in 2 steps; i) HF, H_2O, RT, 12 h, 65%. TIPS = triisopropylsilyl, PPTS = pyridine *p*-toluenesulfonate, DIBAL-H = diisobutylaluminium hydride, Boc = *tert*-butoxycarbonyl.



Scheme 4. Synthesis of an aldehyde **25**, a precursor for aldol reaction, and total synthesis of chiriquitoxin (**2**): a) BnOH, Na_2CO_3, DMF, 150 °C, 2 h, 85%; b) 0.05 N HCl aq., EtOH, RT, 22 h, 51% (recovered starting material 33%); c) DMSO, Ac_2O, RT, 22 h, 96%; d) iminolactone **3**, LDA, LiCl, THF, −20 °C, 40 min, 69%; e) H_2, Pd/C, EtOH, RT, 17 h; f) BocN = C(SMe)NHBoc, $HgCl_2$, Et_3N, DMF, RT, 20 min, 67% in 2 steps; g) MCPBA, CH_2Cl_2, 0 °C, 10 min; h) TFAA, 2,6-lutidine, CH_2Cl_2, 0 °C, 1.5 h; i) NH_3 aq., EtOH, 0 °C, 1 h, 37% in 3 steps; j) HF, H_2O, RT, 14 h; k) TFA, H_2O, 40 °C, 4 days; l) pyridine, H_2O, 35 °C, 12 h, 41% for CHTX (**2**), 28% for 4,9-anhydroCHTX (**32**). Cbz = benzyloxycarbonyl, Bn = benzyl, DMSO = dimethylsulfoxide, LDA = lithium diisopropylamide, TFAA = trifluoroacetic anhydride, TFA = trifluoroacetic acid.

$SnCl_2{\cdot}2H_2O$ in CH_2Cl_2[27] to give 1,2-diol **18** in 66% yield along with the recovered starting material **17** in 15% yield. Cleavage of the 1,2-diol of **18** with sodium periodate and subsequent treatment with PPTS and triethyl orthoformate in ethanol provided intramolecular acetal **19** as a single diastereomer.[28] This route is robust and scalable, which allowed us to synthesize approximately 2 g of **19** to date. Since the product is a fully functionalized intermediate for tetrodotoxin except for guanidine functionality, transformation of **19** into tetrodotoxin was investigated. The guanidine was installed in two steps; 1) deprotection of the *N*-trichloroacetyl group with DIBAL-H[29] and 2) guanidinylation of the resulting amine with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea in the presence of $HgCl_2$, to give di-Boc guanidine **20a** in 69% overall yield. All the protecting groups of **20a** were deprotected with aqueous HF to afford 65% yield of 4,9-anhydrotetrodotoxin (**21**),[30] which could be transformed into tetrodotoxin (**1**) by hydrolysis.[31]

Towards the total synthesis of chiriquitoxin, an indispensable intermediate containing aldehyde at the C-11 position was prepared from **19** as shown in Scheme 4. Since deprotection of the *N*-trichloroacetyl group would be extremely difficult after aldol reaction, the *N*-trichloroacetyl group of **19** was first transformed into benzyl carbamate in one-pot under the conditions developed in this laboratory;[32] heating with benzyl alcohol and Na_2CO_3 in DMF at reflux temperature gave benzyl carbamate **22** in 85% yield. Selective deprotection of the TBS group at the C-11 hydroxy group was best carried out by treatment with aqueous HCl in ethanol to provide primary alcohol **23** in 51% yield along with the recovered starting material **22** in 33% yield. Unexpectedly, oxidation of the primary alcohol to aldehyde proved to be very difficult. Most of the oxidants tested gave low yields of the desired product **24** along with many byproducts including C–C bond cleavaged products. Eventually, Ac_2O/DMSO (Albright–Goldman oxidation) was found to be the sole condition for yielding aldehyde in an ex-

cellent yield. Although the tertiary alcohol was protected as a methylthiomethyl (MTM) ether under the conditions,[33] the resulting aldehyde **25** was used for the next aldol reaction with iminolactone **3**.

To our delight, when the aldol reaction of **25** with the lithium enolate of the iminolactone **3** was carried out in the presence of LiCl in THF at −20 °C according to the methods reported in the literature,[21] the desired *anti*-adduct **26** was obtained as a single product, which was thought to be formed on the basis of the transition state proposed by Xu and co-workers.[21,34] Interestingly, aldol reaction of the aldehyde **24** under the same conditions gave a complex mixture of products, which indicated the importance of the MTM ether.[35]

Installation of the guanidine functionality was straightforward; hydrogenolytic deprotection of the Cbz group and subsequent guanidinylation of the resulting amine with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea in the presence of $HgCl_2$ gave di-Boc guanidine **27**, a synthetic equivalent to chiriquitoxin, in good overall yield. The remaining issue for the total synthesis was removal of all the protective groups of **27**. However, attempted deprotection of the MTM group under the conventional conditions, such as $HgCl_2$ or $AgNO_3$ in aqueous solvents,[36] was unsuccessful. The considerable difficulties encountered at this stage prompted us to develop a new deprotection method of the MTM group based on a different deprotection mechanism. We envisaged that the Pummerer rearrangement of the sulfoxide of a MTM ether would provide a thioacetal intermediate, which collapses to release the corresponding alcohol. In practice, the MTM group of **27** was oxidized with MCPBA, and the resulting sulfoxide **28** (2.2:1 diastereomeric mixture) was treated with trifluoroacetic anhydride and 2,6-lutidine, a condition of Pummerer rearrangement. Hydrolysis of the product **29** with aqueous ammonia provided the desired diol **30** in 37% overall yield in three steps from **27**.

All the other acid-sensitive protective groups of **30** were removed by treatment with aqueous HF, providing 4,9-anhydrochiriquitoxin-13,6-lactone (**31**) and 4,9-anhydrochiriquitoxin (**32**) in a 92:8 ratio (from ^{1}H NMR spectroscopy). The 4,9-anhydro moiety of both compounds was hydrolyzed with 2% TFA/H_2O at 40 °C for 4 days, and then the lactone was hydrolyzed with 0.1% pyridine/H_2O at 35 °C for 12 h to furnish chiriquitoxin (**2**) and 4,9-anhydrochiriquitoxin (**32**) in 41% and 28% yield, respectively, after purification by HPLC on an ion-exchange resin column. The ^{1}H and ^{13}C NMR spectra of the synthesized chiriquitoxin were identical to those of the natural chiriquitoxin.[6] The synthetic $[\alpha]_D$ value ($[\alpha]_D^{31} = -14.6$ ($c = 0.185$, 0.05 N AcOH)) was also consistent with that of reported data ($[\alpha]_D^{25} = -17.3$ ($c = 0.075$, 0.05 N AcOH)).[6]

In conclusion, we have successfully achieved the first total synthesis of chiriquitoxin, the most structurally complex analogue of tetrodotoxin. The success of this total synthesis relied on the new efficient synthetic route of tetrodotoxin from the newly designed intermediate **4**, stereocontrolled aldol reaction of a D-camphor-derived iminolactone for introduction of the glycine unit, and new deprotection procedure of MTM ether by Pummerer rearrangement. This total synthesis confirmed the structure of chiriquitoxin including the configurations of the side chain, and also enables supply of chiriquitoxin and its derivatives for biochemical investigations. Biochemical investigations using the synthesized chiriquitoxin and its intermediate are underway.

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Keywords: chiriquitoxin · natural products · Pummerer rearrangement · tetrodotoxin · total synthesis

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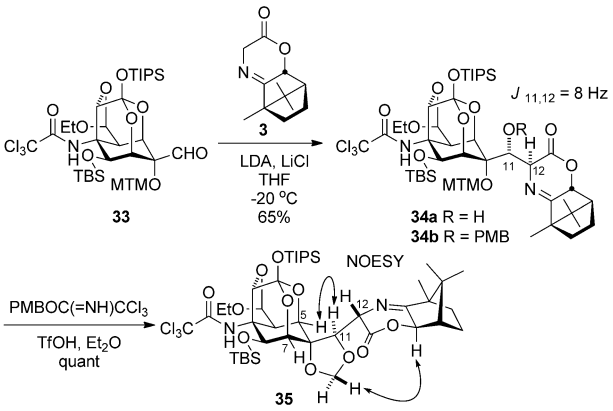
[31] The spectroscopic data of the synthesized 4,9-anhydrotetrodotoxin (**21**) were identical to those of the natural product[30] as well as those reported by our laboratory.[10] Transformation of the 4,9-anhydrotetrodotoxin (**21**) to tetrodotoxin (**1**) under acidic conditions was reported.[5a, 10]
[32] D. Urabe, K. Sugino, T. Nishikawa, M. Isobe, *Tetrahedron Lett.* **2004**, *45*, 9405 – 9407.
[33] Y. Yamada, K. Kato, H. Nagase, Y. Hirata, *Tetrahedron Lett.* **1976**, *17*, 65 – 66.
[34] The stereochemical outcome of the aldol reaction was confirmed by the following experiments; aldol reaction of the trichloroacetyl-protected aldehyde **33** with lithium enolate of the iminolactone **3** under the same conditions provided the aldol adduct **34a** as a single product. Attempted protection of the secondary alcohol at the C-11 position with PMB gave a methylene acetal **35**, instead of the desired PMB ether **34b**. Extensive analysis of the NMR spectra including NOESY determined the configurations of the newly generated asymmetric centers at the C-11 and C-12 positions in the aldol reaction. Since the coupling constants between H-11 and H-12 of both the aldol adducts **26** and **34a** were the same ($J_{11,12}$ = 8.0 Hz), we concluded that the configurations at the C-11 and C-12 positions of **26** are as shown in **26**.



[35] To maximize the synthetic efficiency, an aldehyde possessing guanidine from **20** seemed to be one of the ideal substrate for the aldol reaction. However, the aldehyde prepared from **20b** did not undergo the aldol reaction under the same conditions to give a complex mixture of products.
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