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# Stereocontrolled Total Synthesis of Tetrodotoxin from *myo*-Inositol and D-Glucose by Three Routes: Aspects for Constructing Complex Multi-Functionalized Cyclitols with Branched-Chain Structures

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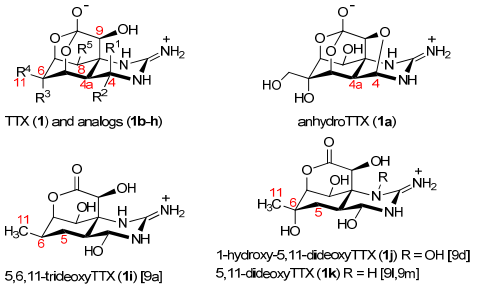
This report describes the stereocontrolled total synthesis of the multi-functionalized cyclitol derivative, tetrodotoxin, containing eight asymmetric carbons and different types of branched-chains, from *myo*-inositol and D-glucose using three different methods. The tetrodotoxin derivatives possess a relatively small molecular weight but unique structural and chemical properties. Selection of the appropriate synthetic method may be useful not only for compounds related to TTX (including related derivatives), but also for other highly complex multi-functionalized cyclitols containing branched-chains.

**Keywords:** Tetrodotoxin, Functionalized cyclitol, Branched-chain sugar, Henry reaction, Ferrier(II) reaction.

Tetrodotoxin (TTX **1**), a well-known marine toxin, was originally isolated from the puffer fish [1]. Four groups reported the structure of TTX in 1964 [2-5]. The original name of TTX was "taricatoxin" (Figure 1).




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| TTX (**1**) [4,5] | H | OH | OH | $CH_2OH$ | OH |
| 4-*epi*TTX (**1b**) [9e] | OH | H | OH | $CH_2OH$ | OH |
| 6-*epi*TTX (**1c**) [9f] | H | OH | $CH_2OH$ | OH | OH |
| 11-deoxyTTX (**1d**) [9f] | H | OH | OH | $CH_3$ | OH |
| 8,11-dideoxyTTX (**1e**) [9g] | H | OH | OH | $CH_3$ | H |
| 11-oxoTTX (**1f**) [9i] | H | OH | OH | CHO | OH |
| 11-norTTX-6(*R*)-ol (**1g**) [9j] | H | OH | H | OH | OH |
| 11-norTTX-6(*S*)-ol (**1h**) [9k] | H | OH | OH | H | OH |

**Figure 1**: Tetrodotoxin (**1**) and its analogs (**1a-k**).

TTX selectively interacts with elements of the sodium channel, inhibiting its activity in the cell membrane [6]. Therefore, TTX is employed as a tool to analyze events that occur *via* the sodium channel [7]. TTX has been isolated from the puffer fish, newts, frogs, octopi, crabs, shellfish, and numerous other aquatic animals. The animals do not produce TTX themselves; instead, it is produced by bacteria such as *Alteromonas* sp., *Vibrio* sp., and *Shewanella* sp. [8]. Yasumoto and others proposed a biosynthetic pathway of TTX and its analogs [9] based on the structure of TTX and its analogs [10]. TTX and its analogs are expected to provide important information in areas such as pharmaceuticals, structure-activity relationships, and biological roles [11]. Therefore, a facile and large-scale synthesis of TTX and its analogs is of great interest. However, preparing modified TTX derivatives from the naturally occurring compound is difficult because of its unique structural and chemical properties (Figure 2) [12].

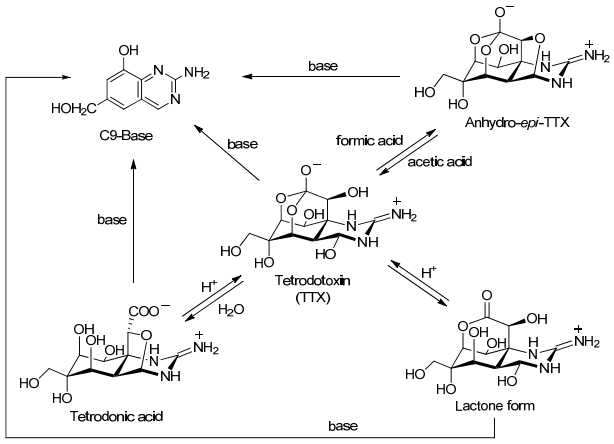



**Figure 2**: Chemical properties of tetrodotoxin.

The total synthesis of TTX and its analogs has presented difficult challenges, despite attempts by numerous research groups [13]. An efficient synthesis of optically active TTX has not been reported in the 30 years following the first total synthesis of (±)-TTX by Kishi *et al*. [14]. In 2003, the groups of Isobe and Du Bois succeeded in synthesizing (–)-TTX, respectively [15a,15d]. Shortly afterward, Isobe's group reported an improved route with fewer steps [15b,15c,15f-i]. In 2005, the total synthesis of (±)-TTX was achieved from *myo*-inositol [16a]; in 2008 and 2010 the synthesis of (–)-TTX from D-glucose was accomplished [16b,16c]. Moreover, Noheda filed a patent application for synthetic methods for TTX and its analogs and intermediates [17]. A review on the synthetic work related to tetrodotoxin appeared in 2011 [18].

This report describes the methods and strategy for the stereocontrolled total synthesis of TTX using three different methods. For the synthesis of TTX, the use of carbohydrates as starting materials was considered for the following reasons. 1) They are inexpensive and readily available. 2) They have asymmetric carbons that can be used synthetically (for example, D-glucose can be used as a 5- or 6-membered compound to enable manipulation in a stereocontrolled manner to direct reaction at a specific position). The "*chiron approach*" [19] can also be employed in the total synthesis of complex natural products using chiral carbohydrates as a starting material. This approach has led to the stereocontrolled introduction of functionalized branched-chains into carbohydrate derivatives as chiral templates [20]. It is noteworthy that the method provides us with construction of a C-C bond bearing various functional groups at branching carbon atoms. Furthermore, the method of introduction allowed stereocontrolled construction of the functionalized branched-chain compounds without considering steric factors of the substrates. Based on these reports, universal methods for stereocontrolled construction of functionalized branched-chains onto carbohydrate derivatives have been developed (Figure 3 and 4) [21,22]. Stereocontrolled introduction of functionalized branched-chains into *myo*-inositol and D-glucose was performed using the developed methods.

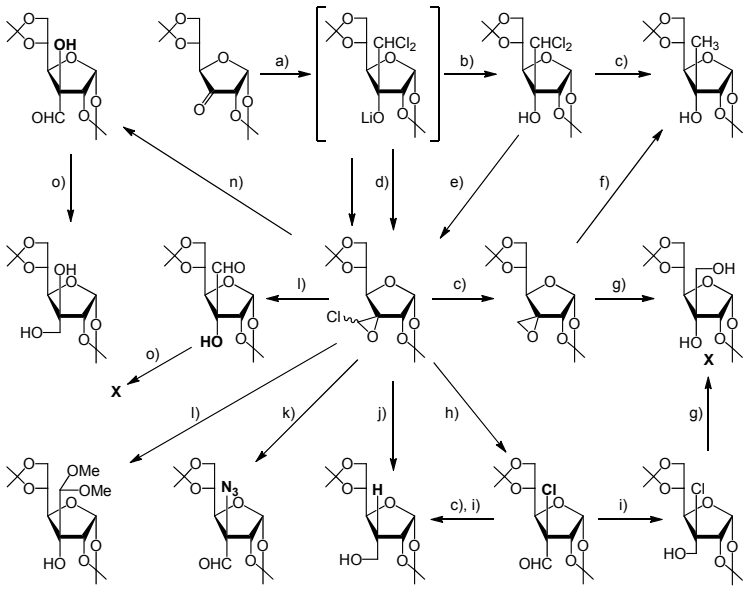
The Henry reaction (nitro-aldol reaction) [23] and the Ferrier(II) reaction [24] were employed for converting the branched-chain sugar into the corresponding branched-chain cyclitol. The Henry reaction has been investigated during synthetic studies of TTX [25] and the Ferrier(II) reaction has been examined for application to the synthesis of branched-chain cyclitol from branched-chain sugars [26]. Based on prior results, the total synthesis was achieved of naturally occurring branched-chain cyclitol compounds, such as cyclophellitol [27], mytilitol, and laminitol [28], starting from D-glucose. The synthetic strategies of TTX from carbohydrates using the three different methods A, B, and C are shown in Figure 5.

**Method A:**
**Stereocontrolled synthesis of (±)-tetrodotoxin from *myo*-inositol** [16a]

The total synthesis of (±)-TTX [29] from *myo*-inositol was achieved using an ortho ester as the protecting agent [30]. The synthetic strategy for (±)-TTX is shown as the retrosynthetic analysis in Figure 6.

The (±)-TTX [29] can be considered chemically equivalent to compound **A**. The cyclic guanidine aminal may be constructed by neighboring group participation of the guanidine moiety and the aldehyde function. The ortho ester structure may be constructed by neighboring group participation of the carboxyl and axially oriented hydroxy groups. The aldehyde function of **A** can be introduced by oxidation of the hydroxymethyl group of **B**. The introduction of the di-*N*-Boc-guanidine moiety was planned for the last stage of the synthesis due to the instability of the intermediates, as anticipated from previous studies [14, 15]. The cyanohydrin group of **B** could be prepared by reaction of the α-azido aldehyde function of **C** with a CN anion. Compound **C** could be synthesized from the corresponding carbonyl compound using dichloromethyl lithium by employing a previously reported method [21]. The asymmetric carbon atoms of TTX, C-5, C-7, and C-8, can be transformed into the C-5, C-3, and C-2 carbon atoms of *myo*-inositol, respectively. This important relation is maintained by the compulsory flip of the stable conformation into an unstable one while protected with an ortho ester.

Following this outline, *myo*-inositol was modified using the following protection and deprotection steps (Scheme 1) [29]. Construction of the branched-chains at C-4 and C-6 of *myo*-inositol, and selective protection of the hydroxy groups occurred following a previously described procedure. Starting compound **3** was synthesized *via* orthoformate **2** from *myo*-inositol [30]. The equatorial hydroxy group of the mono-*O*-benzylated **3** was




*Reagents and conditions*: a) LDA, $CH_2Cl_2$, oxolane, -78 °C. b) $H_2O$, rt. c) *n*-$Bu_3SnH$, AIBN, toluene, reflux. d) 65 °C. e) DBU, DMSO, rt. f) $LiAlH_4$, oxolane. g) NaOH aq, 1.4-dioxane, reflux. h) NaOAc, 15-crown-5, HMPT, 70 °C. i) $NaBH_4$, aq MeOH, rt. j) $NaBH_4$, DMSO, 80 °C. k) $NaN_3$,15-crown-5, HMPT, 70 °C. l) NaOMe, HMPT, 70 °C. m) *n*-$Bu_4NOH$ aq, DMSO, rt. n)CsOAc, 18-crown-6, toluene, reflux. o) $NaBH_4$, MeOH, rt.

**Figure 3**: Methods for constructing various functionalized branched-chain compounds.

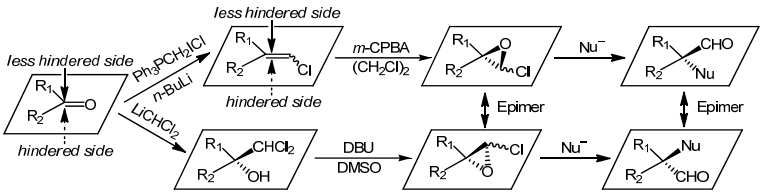



**Figure 4**: Complementary method for constructing functionalized branched-chains.

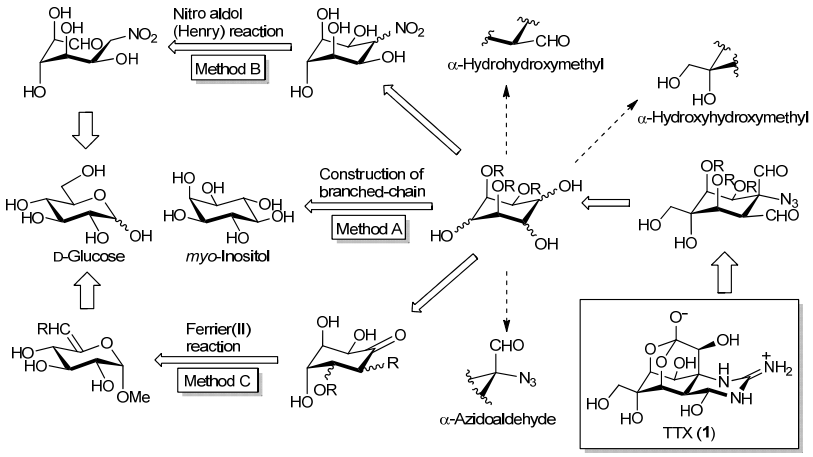



**Figure 5**: Synthetic strategies for TTX from carbohydrates using three different methods.

selectively protected by a methoxymethyl (MOM) group to give **4** in 74% yield. Compound **4** then was oxidized under Swern conditions to give the carbonyl compound **5** in quantitative yield. Addition of lithium diisopropylamide (LDA) and dichloromethane [21] gave the corresponding dichloroethanol derivative **6** as a single stereoisomer in 76% yield. Compound **6** was converted into unstable $\alpha$-hydroxy aldehyde derivative **7** [21], which was immediately reduced with $NaBH_4$ without further purification to give the hydroxymethyl derivative **8** in good yield. The stereochemistry of the quaternary carbon atom in **6** and **8** was confirmed by NOE measurements, as shown in Figure 7. The complete benzylation of **8** to **9**, followed by treatment with 0.1M HCl-MeOH, gave the corresponding tetrol derivative **10** in 97% yield. The treatment of **10** with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and imidazole gave both the desired and the undesired mono-*O*-silylated compounds, **11** and **12**, in 62% and 18% yields, respectively. The structures of **11** and **12** were confirmed by conversion into the corresponding acetates, **11a** and **12a**. Compound **12** was recycled to **11** by de- and reprotection *via* **10**.

The reaction of the *cis*-diol of **11** with 2,2-dimethoxypropane and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) then gave the corresponding acetonide **13** in 93% yield. The conformation of **13** was confirmed by NMR analysis of the corresponding acetyl derivative **13a** ($J_{1,2}$ = 3.1 Hz, $J_{2,3}$ = 7.3 Hz, $J_{1,6}$ = 8.9 Hz, $J_{6,5}$ = 3.1 Hz). Then, **13** was treated with dimethoxymethane and $P_2O_5$ to give the fully protected cyclitol derivative **14** in 75% yield. Removal of the benzyl group of **14** under catalytic reducing conditions gave **15** in 88% yield, which was then transformed into the acetonide **16** in 99% yield. The resulting alcohol **16** was oxidized to give the carbonyl compound **17** in 95% yield. Stereoselective introduction of the hydroxymethyl branched chain at the C-6 position of *myo*-inositol is important in this synthesis. Introduction of the hydroxymethyl branched-chain was first expected to be achieved from Wittig methylation [31] of the carbonyl compound **17** followed by hydroboration-oxidation. However, the alkene **19** was not obtained, likely due to steric hindrance of the MOM and/or

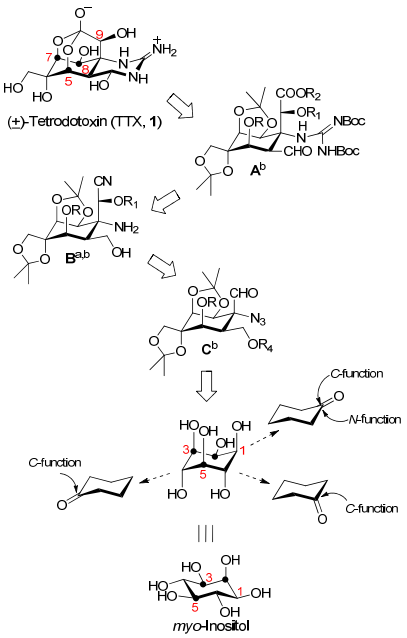



**Figure 6**: Retrosynthetic analysis of (±)-TTX (Method A). (a) The actual conformation of **B** is different: see compound **26**. (b) One of the epimers is drawn.

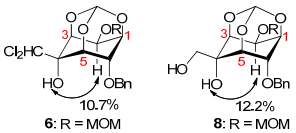
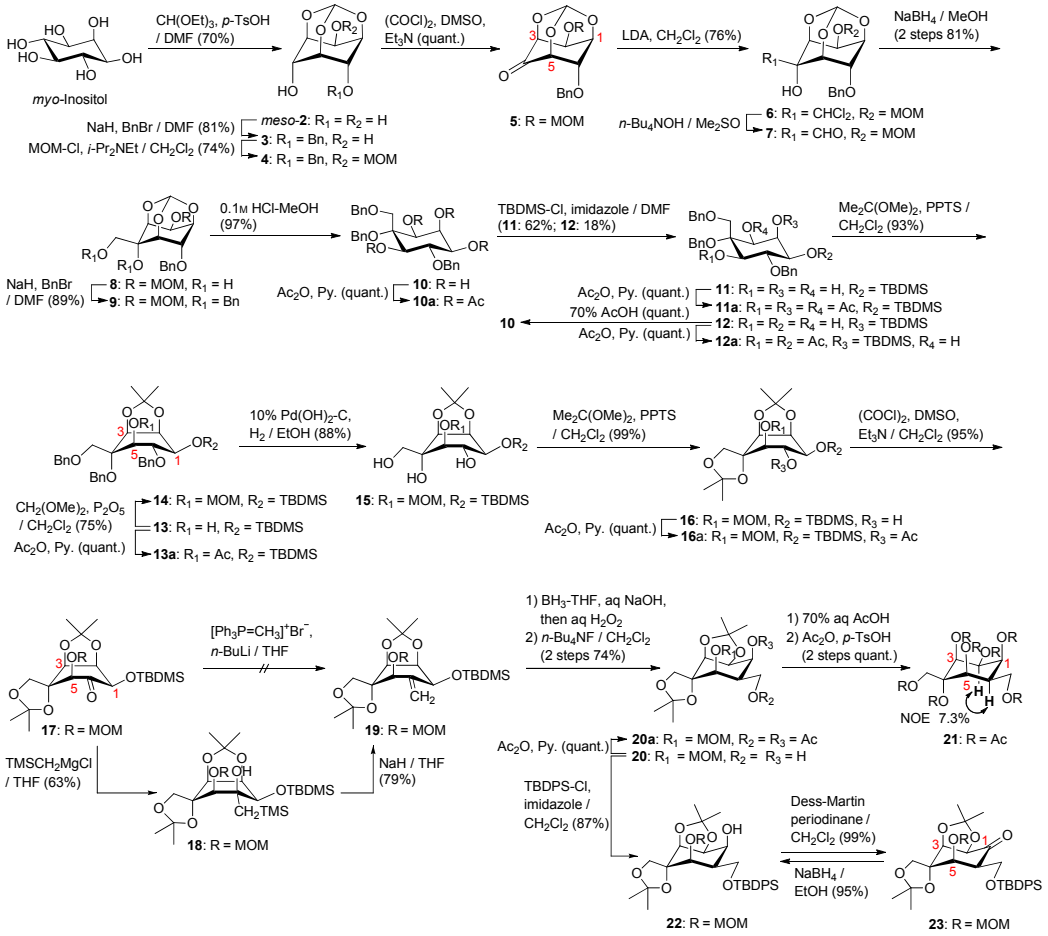


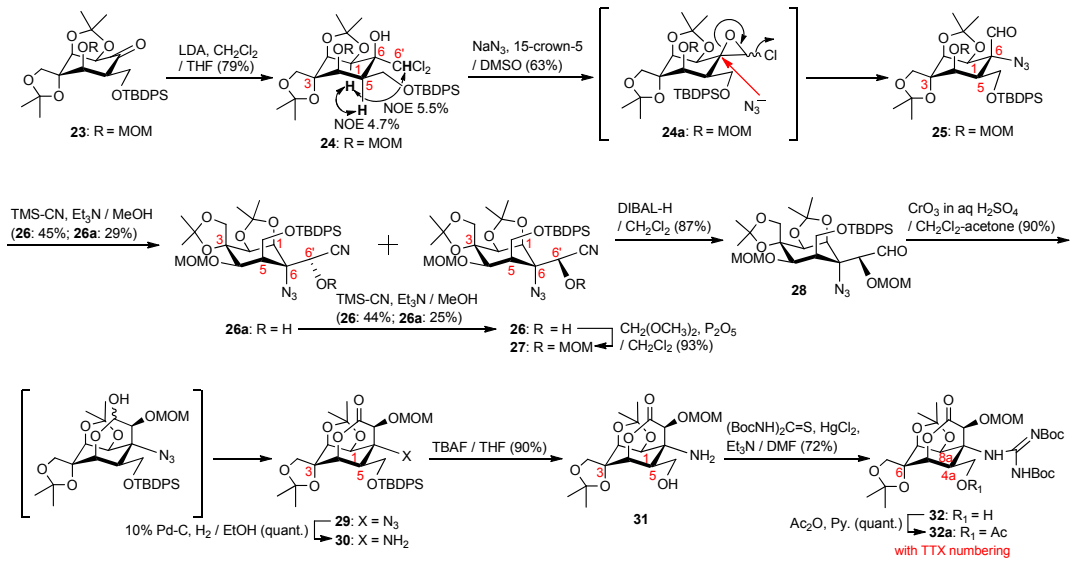

**Figure 7**: NOE correlations of **6** and **8** [29].

TBDMS protecting groups. Peterson's olefination [32] of **17** then was examined to give **19** *via* trimethylsilylmethyl compound **18** in 50% two-step yield. The methylene compound **19** was transformed into hydroxymethyl compound **20** in 74% two-step yield by

hydroboration-oxidation and de-silylation with tetra-*n*-butylammonium fluoride (TBAF). The conformation of **20** and its configuration at the hydroxymethyl branching carbon were confirmed by NMR analysis of its acetylated compound **20a** ($J_{5,6}$ = 5.8 Hz, $J_{6,1}$ = 5.2 Hz) and **21** ($J_{5,6}$ = 3.3 Hz, $J_{6,1}$ = 3.3 Hz, and NOE experiments, shown in Scheme 1). The primary hydroxy group of **20** was selectively protected with a *tert*-butyldiphenylsilyl (TBDPS) group to give the corresponding monohydroxy compound **22** in 87% yield. Compound **22** was then oxidized with Dess-Martin periodinane [33] to give **23** (a key precursor for synthesizing TTX) in 99% yield (Scheme 1).




**Scheme 1**: Synthesis of key intermediate **23** from *myo*-inositol via branched-chain cyclitol derivative **8** on the path to (±)-TTX (with *myo*-inositol numbering) [29].




**Scheme 2**: Synthesis of precursor **32** from **23** on the path to (±)-TTX (with *muco*-inositol numbering) [29].

Introduction of amino function and ortho ester moieties of **B** and **A** was accomplished as follows (Scheme 2) [29]. To construct the α-azido aldehyde branched chain of **C**, **23** was treated with LDA and dichloromethane at −78°C. The expected dichloroethanol derivative **24** was obtained in 79% yield [21]. The stereochemistry at C-6 of **24** was determined by NOE experiments, together with results from the stereoselective reduction of **23** to **22** (Scheme 1, 2). Hence, ketone **23** was reduced with $NaBH_4$ to give only corresponding axial alcohol **22**. The stereoselectivity of the nucleophilic reaction of **23** may be controlled by the 1,3-diaxial relationship of the C-3 and C-5 substituents. The resulting dichloroethanol derivative **24** was then treated with $NaN_3$ in 15-crown-5 ether and DMSO to give the corresponding α-azido aldehyde **25** in 63% yield [21]. The reaction proceeded both stereoselectively and regioselectively. The stereochemistry at C-6 of **25** was verified by X-ray crystallography of cyanohydrin derivative **26** (Figure 8), which was synthesized by reaction of **25** with CN anion as follows. Reaction of α-azido aldehyde **25** and TMS-CN/$Et_3N$ in MeOH gave the corresponding epimeric cyanohydrin derivative **26** [34] and **26a** in 45% and 29% yields, respectively. Careful monitoring of the cyanohydrine reaction by TLC (*n*-hexane-EtOAc, 6:1) suggested that **25** was completely converted into the kinetically controlled product **26a** within 3 min, after which it gradually converted into an equilibrium mixture (3:2) of **26** and **26a**. The more polar product **26** was then treated with dimethoxymethane and $P_2O_5$ to give the corresponding protected methoxymethyl (MOM) derivative **27** in 93% yield.

In contrast, the less polar isomer **26a** could not be protected under these conditions. The undesired isomer **26a** could be transformed into desired isomer **26** by treatment with TMS-CN/$Et_3N$ in MeOH in 44% yield (Scheme 2). The configurations at C-6 and C-6' (C-8a and C-9 with TTX numbering) of **26** were determined by single-crystal X-ray crystallography [34a]. The ORTEP diagram of **26** shows that the configuration of the six-membered ring is the $^3C_6$ form and the configuration at C-6' (C-9 with TTX numbering) is the same as that of natural TTX (Figure 8).

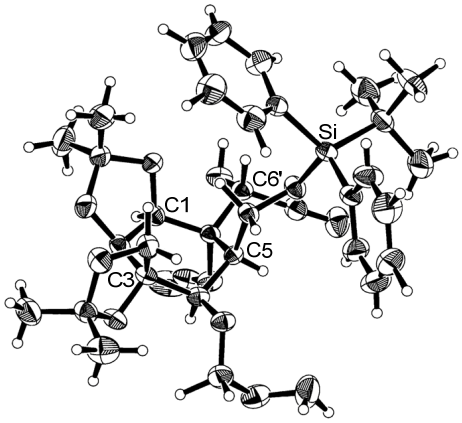



**Figure 8**: ORTEP diagram of **26** with the *muco*-inositol based atomic numbering [19a]. Thermal ellipsoids are drawn at the 30% probability level.

Introduction of ortho ester moiety and guanidine groups to **27** was accomplished as follows (Scheme 2) [29]. Cyanohydrin derivative **27** was treated with diisobutylaluminum hydride (DIBAL-H) to give the corresponding aldehyde derivative **28** in 87% yield. The selective deprotection of the MOM group at O-4 (O-5 with TTX numbering) of **28** and subsequent treatment with Jones' reagent gave the δ-lactone derivative **29** as a single product in 90% yield. The structure of **29** was confirmed by NMR analysis [W-shaped long-range coupling: $J_{5,6'}$ ($J_{4a,9}$ with TTX numbering) = 1.4 Hz, NOESY, and HMBC experiments], as shown in Figure 9. To introduce the guanidine moiety, the azido group of **29** was first reduced with 10% Pd-C and $H_2$ to quantitatively give amino derivative **30**. Then, the TBDPS group of **30** was deprotected using TBAF in 90% yield. Thus, amino derivative **31** was then treated with bis(*tert*-butoxycarbonyl)thiourea, mercury(II) chloride, and triethylamine [35] to give the guanidine derivative **32** in 72% yield. The structure of **32** was confirmed by conversion to the corresponding acetate **32a**.

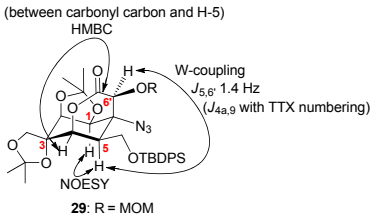
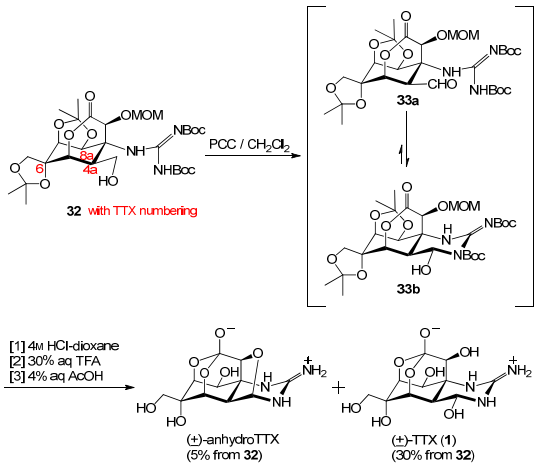



**Figure 9**: NOE correlations of **29** (with *muco*-inositol numbering) [29].

Finally, oxidation of **32** with pyridinium chlorochromate (PCC) and subsequent treatment of the product with 4M HCl-dioxane/MeOH [15a,b], then 30% TFA solution, followed by a 4% aq. AcOH solution, provided a mixture of (±)-TTX, 4,9-anhydro-4-*epi*-(±)-TTX ((±)-anhydroTTX), and other polar decomposition compounds. The mixture was purified by HPLC on a Hitachi-gel #3013-c column ($H^+$ form, 0.05M aq. AcOH) [15a] to give (±)-TTX (including a sight lactone form by $^1H$ NMR), and (±)-anhydroTTX in 30% and 10% yields (from **32**), respectively (Scheme 3). Isomerization of the (±)-anhydroTTX to (±)-TTX in 4% aq. AcOH solution was very slow at temperatures below room temperature. The spectral data for the synthetic (±)-TTX were identical with those of natural TTX [36] ($^1H$ and MS) [9d], [15a,b] (The comparative $^1H$ NMR data are illustrated in Table 1 of Method B).




**Scheme 3**: Final conversion of precursor **32** into (±)-TTX [29].

In the oxidation reaction described above, the signals in the $^1H$ NMR spectrum of the oxidation product were broadened, which made the confirmation of the original aldehyde signals impossible. The results suggested that resulting aldehyde **33a** was interconvertible with the aminal compound **33b**. A similar phenomenon has been reported for 1,2-*O*-isopropylidene-3-*C*-acetaminoethyl-α-D-*xylo*-pentodialdofranose [20a]. Treatment of the other oxidation products (Dess-Martin [33], Swern, or 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO) [37] oxidation products) under acidic conditions (in Scheme 3) did not provide either (±)-TTX or (±)-anhydroTTX. Some side reactions must

have occurred during oxidation of the primary hydroxy group of **32**. These oxidation products showed different $R_f$ values on TLC compared with those of **33a** (**33b**). The determination of its structure was difficult because of this broadened $^1$H NMR spectrum.

In conclusion, the total synthesis of (±)-TTX from *myo*-inositol was accomplished in a highly stereocontrolled fashion and in excellent yield (34 steps to **1** from *myo*-inositol). Key steps included 1,3,5-orthoformylation of *myo*-inositol with conformational inversion to differentiate the remaining three free OH groups. These groups were oxidized in a stepwise manner to the carbonyl function, followed by specific nucleophilic addition, including successive spiro α-chloroepoxide formation and its ring-opening with an azide anion, to give the desired branched-chain structure.

**Method B:**

**Stereoselective and efficient total synthesis of optically active tetrodotoxin from D-glucose** [16b]: The total synthesis of optically active (–)-TTX from D-glucose was achieved using the Henry reaction [23] as the key transformation. The synthetic strategy for **(–)**-TTX is shown as a retrosynthetic analysis in Figure 10. The retrosynthetic plan for the synthesis of (–)-TTX from D-glucose focuses on preparation of the key intermediate, ketone **D** (**23**). This intermediate was successfully converted to TTX [16a] as mentioned in method A (Scheme 1-3). In method A, (±)-TTX was synthesized *via* compounds **A'**, **B'**, **C'**, and **D**. Therefore, the synthesis of optically active compound **D** from D-glucose was a promising route to the synthesis of (–)-TTX. Compound **D** can be synthesized from compound **E** through the Henry reaction, as previously described [25]. The key intermediate, **E**, was synthesized using standard reactions from D-glucose, as described below (Scheme 4-6).

3-*C*-Hydroxymethyl-1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*-hexofuranose (**36**) [20a] was prepared by stereoselective *m*-chloroperbenzoic acid (*m*-CPBA) oxidation of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-methylene-α-D-*ribo*-hexofuranose (**34**) [38] derived from D-glucose, followed by hydrolysis with 1M aq. NaOH. The two hydroxy groups of **36** were protected with a benzyl (Bn) group to give 3,3'-di-*O*-benzyl derivative **37** in 95% yield. Compound **37** then was selectively hydrolyzed using 70% aq. AcOH solution at room temperature to give the 5,6-diol **38** in quantitative yield. Oxidative degradation of **38** with $NaIO_4$ in aq. MeOH solution gave the corresponding aldehyde **39**, which then was treated with nitromethane in methanol in the presence of NaOMe to give nitro alcohol **40** (diastereomer ratio = *ca.* 10:1) in 75% two-step yield. The diastereomeric mixture of nitro alcohol **40** was treated with methanesulfonyl chloride in the presence of triethylamine to give the corresponding single nitro-olefin **41** (**G** in Figure 10) in 85% yield. Reaction of **41** with *n*-butyl lithium and bis(phenylthio)methane gave the branched bis(phenyl)dithioacetal derivatives **42a** and **42b**. The ratio of **42a**:**42b** (*ca.* 10:1) was determined by $^1$H NMR (comparison of the intensities of H-5) of the crude mixture. The addition of dithioacetal anion proceeded with greater stereoselectivity (10:1) than that described in a previous report [25a] using 1,3-dithiane (5:4). The greater stereoselectivity might be due to the bulkiness of the bis(phenylthio)methane reagent. The desired compound **42a** was isolated by fractional crystallization in 80% yield. The configuration at C-5 of **42a** was determined by derivation into the corresponding cyclitol compound **44a**, as described below. The 1,2-*O*-isopropylidene group of **42a** was hydrolyzed upon treatment with 85% aq. AcOH solution under reflux. The resulting nitro-sugar **43** (**F** in Figure 10) was treated with 1.5 mol eq of $NaHCO_3$ in aq. MeOH solution to give crystalline *muco*-inositol derivative **44** [39] (**E** in Figure 10) in 84% two-step yield.

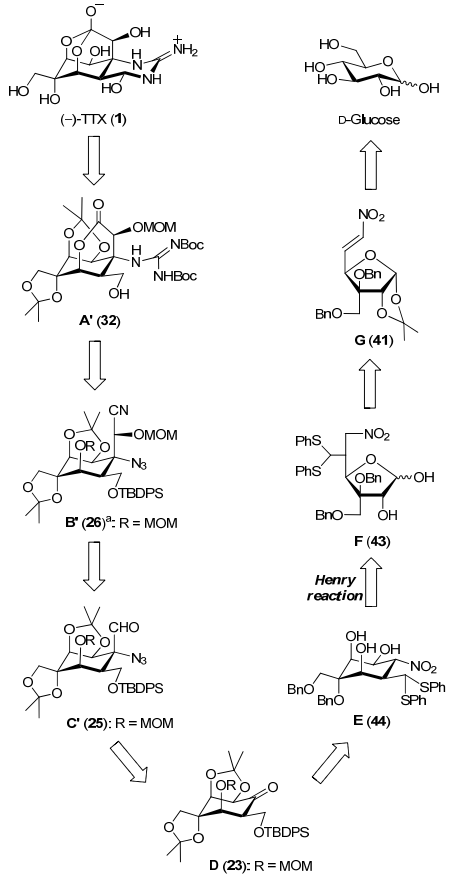
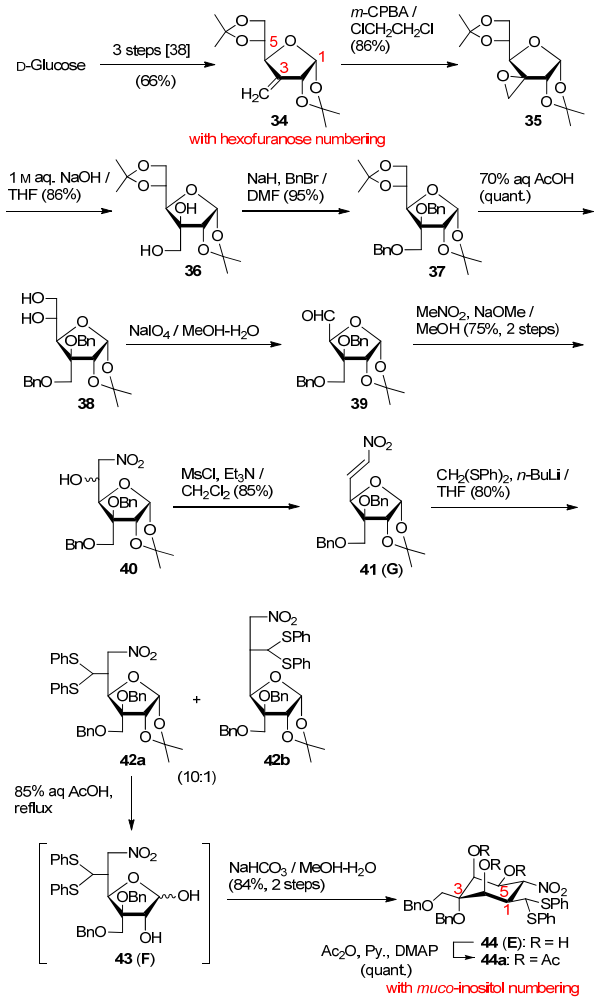



**Figure 10**: Retrosynthetic analysis of (–)-TTX from D-glucose (Method B). (a) The actual conformation of **B'** is different: see compound **26**.

Acetylation of **44** under standard conditions gave tri-*O*-acetate **44a**, quantitatively. The coupling constant for **44a** ($J_{1,2}$ = 2.5 Hz, $J_{4,5}$ = 3.5 Hz, $J_{1,6}$ = $J_{5,6}$ = 11.0 Hz) suggested the configuration for C-5 (C-8 with TTX numbering). The formation of four stereoisomers, including **44**, is possible from the Henry reaction, but the three undesirable stereoisomers were not isolated. This stereoselectivity is likely to be due to the stable six-membered cyclic transition state of intermediate [40].

Reaction of the *vicinal*-diol of **44** with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) gave the corresponding acetonide **45** in 86% yield. Acetonide **45** then was treated with dimethoxymethane and $P_2O_5$ to give the fully protected cyclitol **46** in 93% yield. Treatment of **46** with *N*-bromosuccinimide (NBS) in aq. MeCN solution gave the unstable aldehyde **47**, which was immediately reduced with $NaBH_4$ to give the hydroxymethyl derivative **48** in 82% two-step yield. Silylation of **48** with TBDPS-Cl and imidazole gave the fully protected nitro cyclitol **49** in 95% yield (Scheme 5).

Transformations of the nitro derivative into the carbonyl derivative were examined. Initially, compound **49** was treated with potassium *tert*-butoxide (*t*-BuOK) and *m*-CPBA in benzene [41a] to give decomposed polar compound(s) and a minor amount of the desired product **50** (*ca.* 5% yield). Some side reactions (mainly retro-aldol reaction and subsequent decomposition under basic conditions) proceeded during oxidation of the nitronate intermediate. Therefore, McMurry's transformation [41b] from the nitronate to the carbonyl was applied. Compound **49** was treated with *t*-BuOK, and the resulting nitronate was subjected to ozonolysis ($O_3$) in toluene at

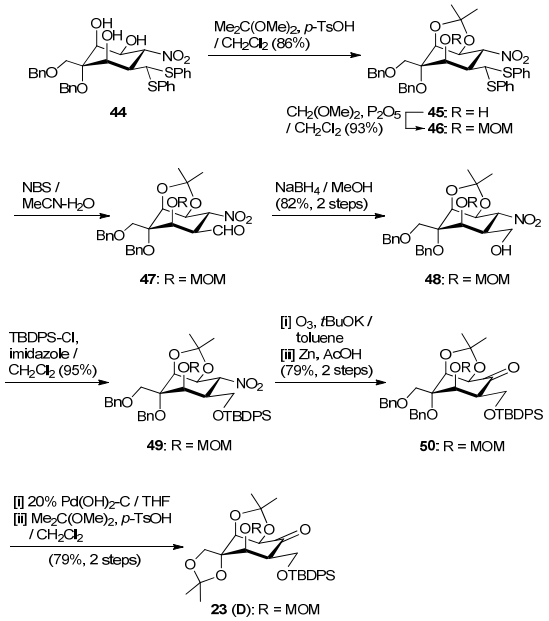



**Scheme 4**: Synthesis of nitro cyclitol **44** (**E**) using the inter- and intra-molecular Henry reaction.

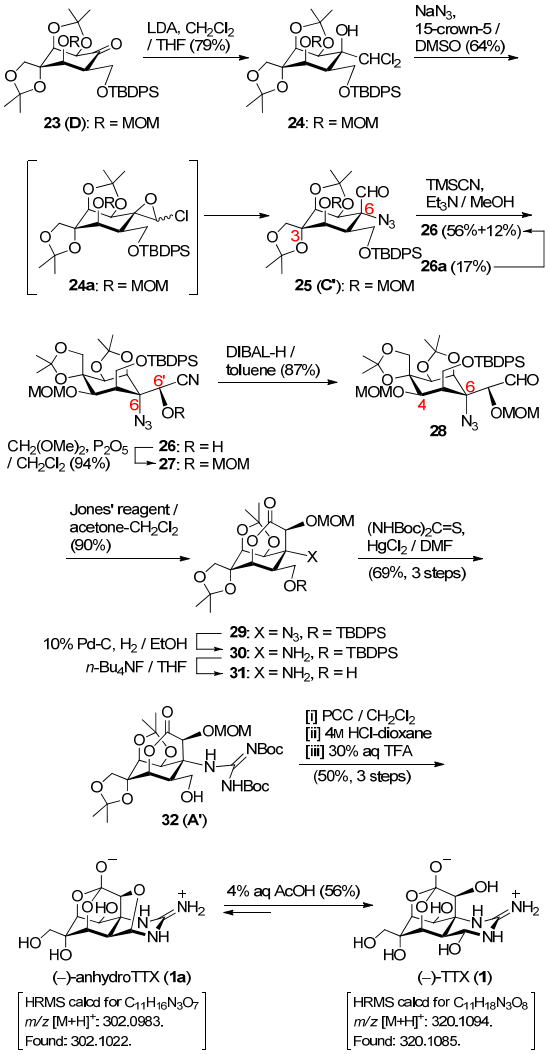
–78°C to give ketone **50** in moderate yield (79%). The transformation of **50** into compound **23** (**D** in Figure 10) was achieved by catalytic reduction, and subsequent acetonide protection of the resulting diol in 79% two-step yield (Scheme 5).

Further conversion of ketone **23** into (–)-TTX was conducted using an established route for (±)-TTX synthesis, including successive spiro α-chloroepoxide formation and its ring-opening with azide anion [21, 22] as a key step.

To obtain compound **25**, ketone **23** was treated with LDA and dichloromethane at –78°C to give the expected dichloroethanol derivative **24** as a single isomer in 79% yield. Dichloroethanol derivative **24** was treated with $NaN_3$ and 15-crown-5 ether in DMSO at 75°C to give the α-azido-aldehyde **25** (**C'** in Figure 10) *via* spiro α-chloroepoxide derivative **24a** in 64% yield, with complete stereo- and regio-selectivity. The structure of **25**, including the stereochemistry at C-6 (C-8a with the corresponding TTX numbering), was determined by X-ray structure analysis. The reaction of α-azido-aldehyde **25** and TMS-CN/$Et_3N$ in MeOH gave the corresponding cyanohydrin **26** and its epimer **26a** in 56% and 17% yields, respectively. Stereochemistry at C-6' (C-9 with the corresponding TTX numbering) of **26** was the same as that observed in the total synthesis of (±)-TTX. The undesired isomer **26a** could be isomerized into the desired isomer **26** under the same reaction conditions. After two cycles of this procedure, the total yield of **26**




**Scheme 5**: Synthesis of optically active key intermediate **23** (**D**).




**Scheme 6**: Conversion of optically active ketone **23** (**D**) into (–)-TTX (**1**) (with *muco*-inositol numbering).

was 68%. To introduce amino and ortho ester moieties, the hydroxyl group of **26** was protected with the methoxymethyl (MOM) group, and subsequently treated with DIBAL-H to give the corresponding aldehyde **28** in 82% two-step yield from **26**. Selective deprotection of the MOM group at O-4 (O-5 with the corresponding TTX numbering) of **28** and subsequent treatment with Jones' reagent gave the δ-lactone **29** as a single product in 90% yield. Monitoring of the reactions of aldehyde **27** using TLC (*n*-hexane/ethyl acetate 4:1) suggested that the deprotection of the MOM group first formed the hemiacetal intermediate, then immediately oxidized into δ-lactone **29**. To introduce the guanidine moiety, the azido group of **29** first was reduced with 10% Pd-C and $H_2$ to give the amine **30** in quantitative yield. Subsequent deprotection of the TBDPS group using TBAF gave alcohol **31**. Guanidinylation [35] of **31** provided the precursor **32** (**A'** in Figure 10), which was converted to 4,9-anhydro-4-*epi*-TTX (anhydroTTX, **1a**) by oxidation with pyridinium chlorochromate (PCC), followed by treatment with 4M HCl-dioxane/MeOH, [15a,b] and then 30% aq. $CF_3COOH$ in 50% yield. Isomerization of **1a** by treatment with 4% aq. AcOH at 60°C for two days yielded a 1:4 mixture of **1a**:**1**. The mixture was purified by HPLC [15a] to give **1** in 56% yield (Scheme 6). The spectral data ($^1$H, MS) for the synthetic **1** agreed well with those of natural TTX [9d,15a,b,36] (Table 1 and Figure 11). Moreover, specific rotations of synthetic **1a** and **1** were $[\alpha]_D^{28}$ +1.2 (*c* 0.18, 3% aq. AcOH) and $[\alpha]_D^{28}$ –3.75 (*c* 0.13, 3% aq. AcOH), respectively. Both rotation values agreed well with those reported by Isobe *et al.* [15a-c,e].

**Table 1**: Comparative $^1$H NMR spectroscopic data of (–)-TTX (**1**) [9f].

| Position | Synthetic TTX[a] | Natural TTX [9f] | Synthetic anhydroTTX |
|---|---|---|---|
| 4 | 5.50 (d, *J* = 8.9 Hz) | 5.50 (d, *J* = 9.4 Hz) | 5.53 (s) |
| 4a | 2.35 (d, *J* = 9.6 Hz) | 2.35 (d, *J* = 9.5 Hz) | 2.94 (d, *J* = 2.6 Hz) |
| 5 | 4.25 (br s) | 4.25 (br s) | 4.36 (dd, *J* = 2.2, *J* = 2.6 Hz) |
| 7 | 4.09 (t, *J* = 2.1 Hz) | 4.08 (t, *J* = 1.8 Hz) | 4.17 (t, *J* = 2.2, d, *J* = 2.6 Hz) |
| 8 | 4.30 (d, *J* = 2.1 Hz) | 4.30 (d, *J* = 1.5 Hz) | 4.63 (d, *J* = 2.2 Hz) |
| 9 | 3.96 (s) | 3.96 (s) | 4.58 (s) |
| 11 | 4.01 (d, *J* = 12.4 Hz) | 4.02 (d, *J* = 12.6 Hz) | 4.00 (d, *J* = 12.2 Hz) |
| | 4.06 (d, *J* = 12.4 Hz) | 4.04 (d, *J* = 12.6 Hz) | 3.96 (d, *J* = 12.2 Hz) |

(a) 600 MHz, in 3% $CD_3COOD/D_2O$, referenced to $CHD_2COOD$ (2.06 ppm).

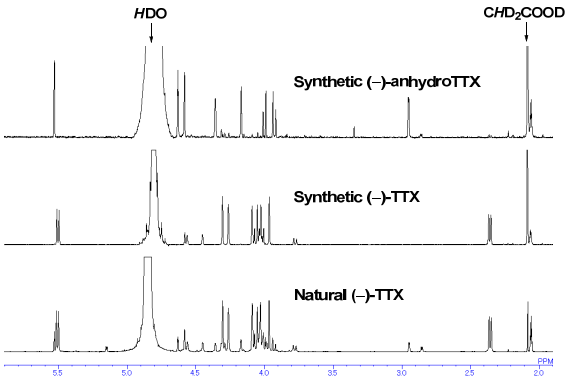



**Figure 11**: $^1$H NMR spectra of (–)-TTX (**1**) and (–)-anhydroTTX.

In conclusion, the total synthesis of optically active (–)-TTX was accomplished from D-glucose using the Henry reaction as a key framework in a highly stereoselective manner (34 steps to **1** from D-glucose).

**Method C:**

**Total synthesis of (–)-tetrodotoxin from D-glucose: A route to multi-functionalized cyclitol using the Ferrier(II) reaction** [16c]: Another route was used for synthesizing (–)-TTX from D-glucose using the Ferrier(II) reaction [24] for key skeleton construction. The synthetic route to (–)-TTX was based on prior experience with the total synthesis of (±)-TTX from *myo*-inositol [16a]. Total syntheses using methods A and B were conducted successfully using the common intermediate **D** (**23**), eventually in an enantiospecific manner [16b]. The synthetic strategy for (–)-TTX is shown as a retrosynthetic analysis in Figure 11. Compound **D** (**23**) was derived from D-glucose through multi-functionalized cyclitol **I** [16a,b], which can be prepared by reaction of enol acetate **J** *via* the Ferrier(II) reaction [24]. Compound **J** was obtained by standard transformations of D-glucose using stereoselective introduction of a branched chain at C-3 with D-glucose (C-6 with the corresponding TTX numbering). The transformation of **D** into (–)-TTX, *via* compounds **C'**, **B'**, and **A'**, was accomplished in a manner similar to that used for methods A and B [16a,b].

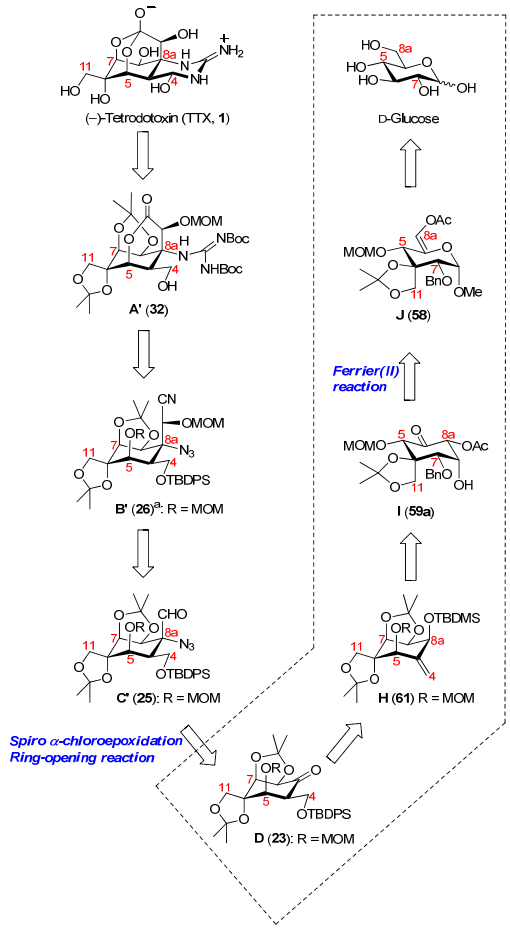
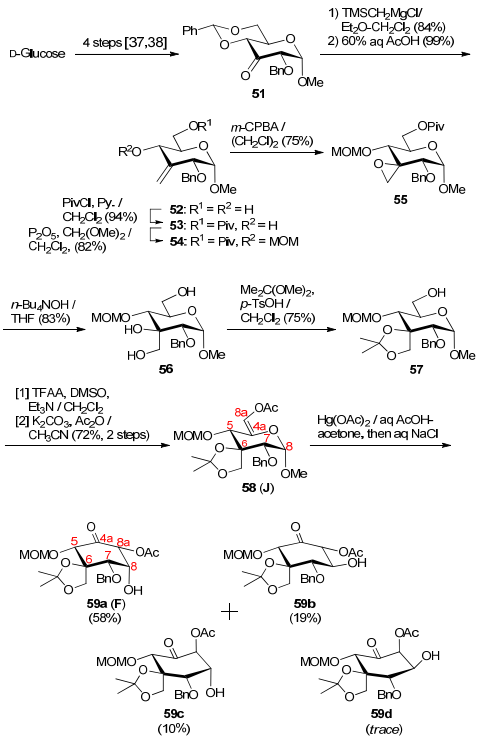



**Figure 12**: Retrosynthetic analysis of (–)-TTX from D-glucose (Method C). (a) The actual conformation of **B'**, **I**, and **H** is different: see compound **26, 59a**, and **61** with the corresponding TTX numbering.

The total synthesis of (–)-TTX from D-glucose is shown in Schemes 7 and 8. Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosid-3-ulose (**51**) was prepared by regioselective benzylation followed by Swern oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside derived from D-glucose. Peterson's olefination [42] of **51**, followed by treatment with aq. AcOH solution, gave methylene derivative **52** *via* a trimethylsilyl derivative. Regioselective protection of **52** with pivaloyl and methoxymethyl groups gave **54**, which was oxidized using *m*-CPBA *via* a stereochemical course reported previously [43] to afford epoxide **55**. Alkaline hydrolysis of **55** yielded triol **56**, which was then converted into the acetonide **57**. Oxidation of **57** resulted in an unstable aldehyde, which was immediately converted into enol acetate **58** (**J** in Figure 12) as a single geometrical isomer. The (*Z*)-configuration at C-8a of **58** was determined by NOESY analysis

(Figure 13). The key reaction of the intramolecular cyclization [Ferrier(II) reaction] of enol acetate **58** involved several mercury reagents in aq. AcOH-acetone solution at room temperature. Results are shown in Table 2. As a result, the desired stereoisomer **59a** (**I** in Figure 12) was obtained in 58% yield using $Hg(OAc)_2$ (**59a**:**59b**:**59c**:**59d** = 6:2:1:0) under the conditions listed in entry 4. In contrast, Ferrier(II) reaction using $PdCl_2$ [24b] afforded a complex mixture. The ($\pi$-allyl)palladium complex reaction intermediate was not smoothly generated, and the starting enol acetate gradually decomposed during heating. Thus, $Hg(OAc)_2$ was chosen as the most suitable reagent for this enol acetate **58**. Structures of the four stereoisomers **59b-d** were confirmed by $^1$H NMR analysis. The chemical shifts and $J$ values of **59a** suggested the structure in Figure 13 (H-8a, 5.21, $J_{8a,8}$ = 3.3 Hz; H-8, 4.21, $J_{8,7}$ = 2.2 Hz, $J_{8,OH}$ = 1.5 Hz; H-4, 4.02, $J_{7,5}$ = 0 Hz; H-5, 4.31, $J_{8a,5}$ = 1.0 Hz). Furthermore, the configurations at C-6 and C-8a of **59a** with the corresponding TTX numbering were verified by single-crystal X-ray crystallography of **61** (Figure 14). Efforts to transform **59b-d**




**Scheme 7**: Synthesis of multi-functionalized cyclitol derivative **59** toward (–)-TTX using the Ferrier(II) reaction (with the corresponding TTX numbering).

**Table 2**: Ferrier(II) reaction conditions and product yields.

| Entry | Reagent | Time (h) | Ratio of isomer 59[a] a : b : c : d | Isolated Yield of 59a |
|---|---|---|---|---|
| 1 | $Hg(OCOCF_3)_2$ | 0.3 | 6 : 0 : 1 : 3 | 33% |
| 2 | $HgCl_2$ | 4 | 0 : 0 : 1 : 2 | *trace* |
| 3 | HgO | 5 | 5 : 0 : 2 : 1 | 48% |
| 4 | $Hg(OAc)_2$ | 1.5 | 6 : 2 : 1 : 0 | 58% |

(a) Determined by $^1$H NMR spectrum.

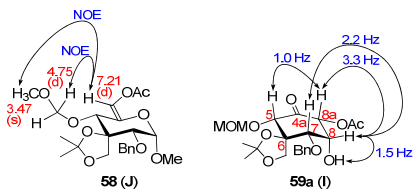
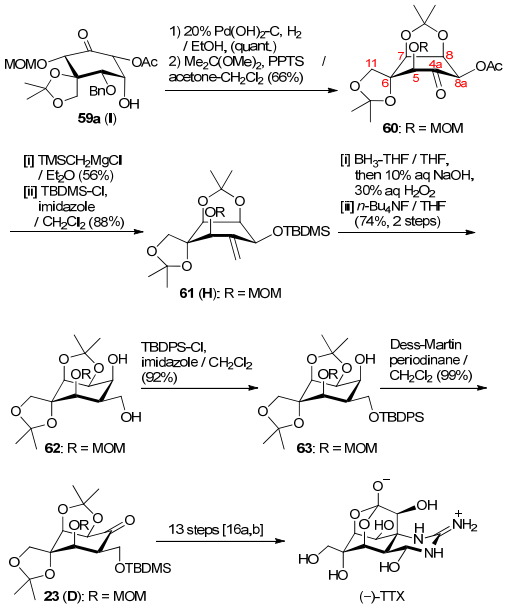



**Figure 13**: Structural assignments of enol acetate **58** and cyclitol **59a** (chemical shifts, spin-spin coupling pattern, NOE correlation of **58** and $J$ values of **59a** with the corresponding TTX numbering).




**Scheme 8**: Total synthesis of (–)-TTX from multi-functionalized cyclitol derivative **59a** (with the corresponding TTX numbering).

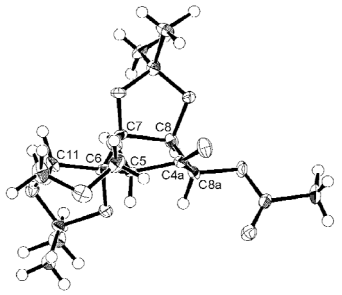



**Figure 14**: ORTEP drawing of **61** with the corresponding TTX numbering. Thermal ellipsoids were scaled to enclose 30% probability.

into **59a** were unsuccessful. Some side reactions, such as β-elimination, occurred during the stereochemical inversion of hydroxy substituents at C-8 (Scheme 7).

Protected diol **60** was obtained by catalytic debenzylation of **59a**, followed by isopropylidenation. Compound **60** was then transformed into **61** (**H** in Figure 12) by Peterson's olefination [42] and subsequent *O*-silylation. Methylene derivative **61** was converted into the corresponding hydroxymethyl derivative **62** by hydroboration-oxidation and de-*O*-silylation. Selective protection of the primary hydroxy group of **62** using TBDPS group afforded the corresponding monohydroxy compound **63**, which was then oxidized with Dess-Martin periodinane [33] to give optically active ketone **23** (**D** in Figure 12), a key intermediate in the synthesis of TTX (Scheme 8). Further reactions of **23** transformed it into (–)-TTX along an established route [16a], including successive spiro $\alpha$-chloroepoxide formation and its ring-opening with an azide anion [21,22] as a key step. Spectral data ($^1$H and MS) for the synthetic TTX agreed well with those for natural TTX [15a,b,16a,b] (same as method B). The total synthesis of (–)-TTX from D-glucose using the Ferrier(II) reaction as the key skeleton construction reaction was achieved successfully using effective and stereoselective steps (35 steps to **1** from D-glucose).

In studies of TTX synthesis, the total synthesis of TTX by three routes (method A, B, and C) was accomplished [16a,16b,16c]. These methods provide a choice for synthesizing compounds related

to TTX (including related derivatives), as well as other highly complex multi-functionalized cyclitols bearing branched-chain structures such as cyclophellitol [27], mytillitol, and laminitol [28].

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