JOC The Journal of Organic Chemistry

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A Convergent Approach to the Dioxaadamantane Core of (±)-Tetrodotoxin§

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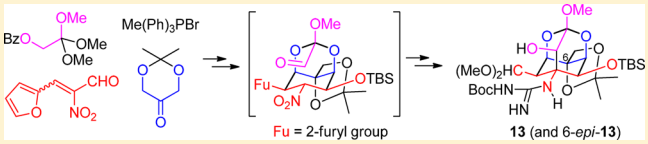
Supporting Information

ABSTRACT: A fully stereocontrolled 1,3-diol orthoesterification and a water-promoted intramolecular Henry addition, combined with the previously reported formal (3 + 3) annulation of α-nitro-α,β-enals and 2,2-dimethyl-1,3-dioxan-5-one, provided for a short convergent pathway to the dioxaadamantane core of (±)-tetrodotoxin.



INTRODUCTION

With more than 32 000 references since 1910 and a steady increase of about 600 additional references per year for the past decade, tetrodotoxin (TTX, Scheme 1, the poison of the fugu fish) is a compound familiar to the scientific community, in particular to organic chemists and neurobiologists.[1] Tetrodotoxin's most significant property, its ability to block voltage-gated sodium channels in a most efficient and selective manner, was instrumental for the identification, isolation, and purification of the main protein subunit of such channels[2] and continues to be the focus of much interest.[3]The pharmacological significance of tetrodotoxin, already put forward by Mosher in 1986,[4] is currently being studied in the fields of analgesia (for severe cancer and neuropathic opioid-refractive pain relief), drug-addiction withdrawal treatment, and local anesthesia.[5]

For chemists, the toxin was initially a challenging structural problem,[6] and afterward a highly demanding synthetic goal, which was realized first as a racemic mixture by Kishi and co-workers[7,8] and later in enantiopure form by the groups led by Isobe, Du Bois, Noheda, and Sato.[9−11] We recently achieved a formal synthesis of racemic tetrodotoxin from furfural[12] through α-nitroenal **2**,[13] protected nitrocyclitol **3**,[12,14] and Sato's intermediate **4** (Scheme 1).[9e]

We herein describe the successful implementation of a new approach to the dioxaadamantane core of tetrodotoxin, which combines the previously reported formal (3 + 3) annulation of α-nitro-α,β-enals and 2,2-dimethyl-1,3-dioxan-5-one (**2** → **3**, Scheme 1),[14a,b] with a fully stereocontrolled 1,3-diol orthoesterification (a process where the ortho-acid function that tetrodotoxin has at C10 is built in the form of an orthoester embracing the oxygens at C5 and C7 of **3**, as shown in **6**) and a water-promoted intramolecular Henry addition (**6c** → **7**, Scheme 1; a transformation where the nitro group, a critical element in the reported annulation process of **2** into **3**, was further used with advantage to promote the C8a−C9 bond formation). We also report the transformation of **7** into intermediates **13** and 6-*epi*-**13** endowed with all the functionality and relative stereochemistry required by (±)-tetrodotoxin and its 6-epi-analogue, respectively.[15] So far, our attempts to fully deprotect **13** and 6-*epi*-**13** have met with failure; additional studies are required to tune the new synthetic approach and make it apt to reach TTX.

RESULTS AND DISCUSSION

Conversion of **3** into ortho esters of type **6** was initiated with protection of its C8-hydroxyl group as a TBS ether, followed by exomethylenation at C6 (Scheme 2). This last operation removed the C6 keto group (thus preventing any epimerization at positions 5 and 7 to take place when opening the acetal bridge, step 6, Scheme 2) and replaced it with a carbon−carbon double bond, hence leaving the system ready for the posterior formation of the diol unit the toxin has at positions 6 and 11 (step 12, Scheme 3).

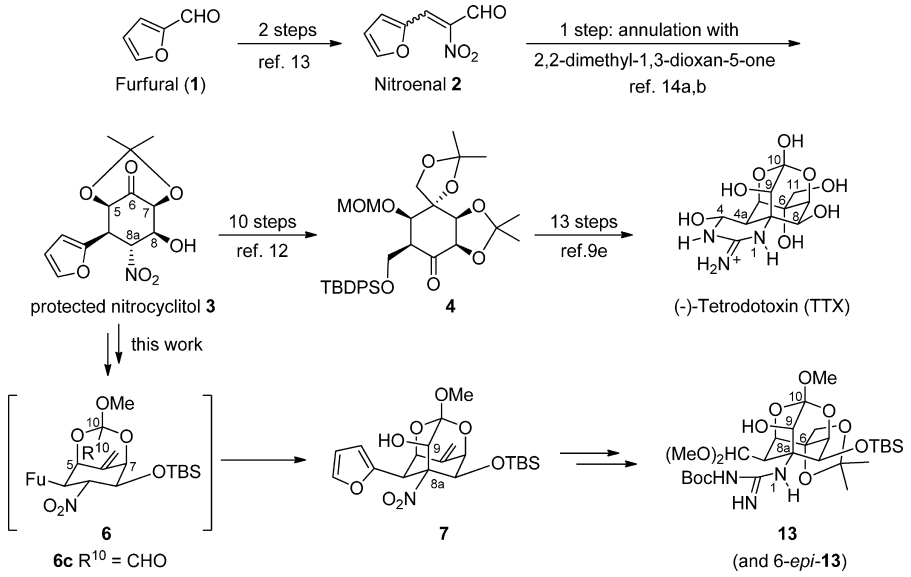
After acid hydrolysis of the isopropylidene acetal, a two-carbon fragment, precursor of C9 and C10 of TTX, was attached to the two free hydroxyl groups of the resulting diol **5** in the form of a methyl ortho ester. Thus, heating of **5** with 2,2,2-trimethoxyethyl benzoate (itself prepared from chloroacetonitrile)[16] and *p*-TsOH gave **6a** (isolated in 67% yield as a single stereoisomer), in which C10 was generated with the stereochemistry appropriate to later address the closure of the dioxaadamantane unit. This outcome was expected, since orthoesters of type **6** should be substantially more stable than their epimers at C10 (and hence the major reaction products under thermodynamic conditions) because at their most stable conformation[17] they would enjoy a double anomeric effect[18] and display the sterically most demanding R^{10} substituent in a less-crowded location.[19]

The hydrolysis of the benzoate group in **6a** was most effective using a 3% solution of NaOMe in MeOH, and the oxidation of the resultant alcohol **6b** to the desired aldehyde **6c**

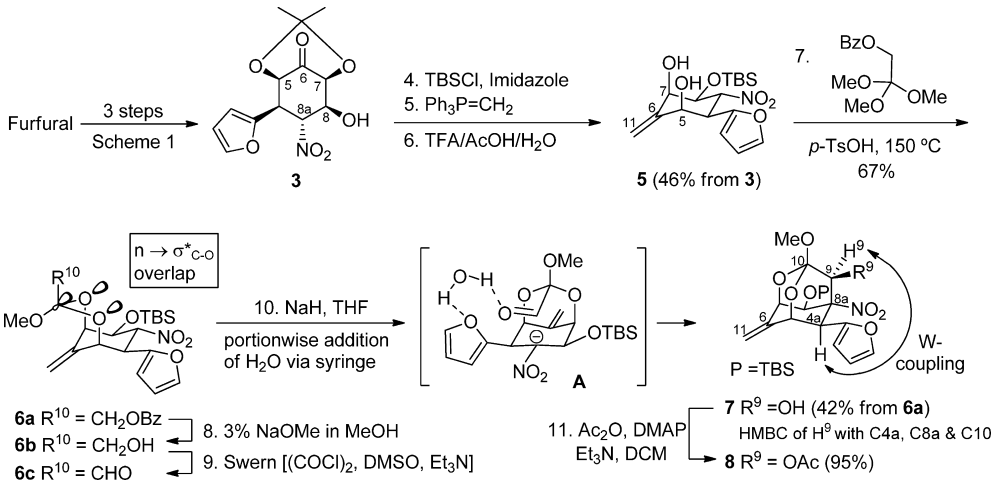
Received: July 8, 2013
Published: April 15, 2014

   dx.doi.org/10.1021/jo500056w | J. Org. Chem. 2014, 79, 4300−4305

Scheme 1. Synthetic Studies from Furfural toward Tetrodotoxin through Protected Nitrocyclitol 3



Scheme 2. Synthetic Sequence Used To Build the Dioxaadamantane Unit from Nitrocyclitol 3



took place efficiently under Swern conditions. Both **6b** and **6c** proved to be unstable and were used in crude form, as obtained.

Besides base (NaH in dry THF), the key intramolecular nitroaldol reaction of **6c** required the addition of very small amounts of water to the reaction mixture until disappearance of the starting aldehyde. Preliminary density functional theory (DFT) calculations revealed a lowering of the energy barrier for the nitroaldol addition when one molecule of water was incorporated in the calculation.[20] The interaction of water with the carbonyl and furyl groups (as illustrated in **A**, Scheme 2) would facilitate the reaction while appropriately orienting the carbonyl oxygen away from the OTBS group. This would also explain the formation of the *β*-hydroxy nitro derivative **7** as the single (desired) stereoisomer shown, which was isolated with an overall yield of 42% for the last three steps from **6a**: benzoate hydrolysis, alcohol oxidation, and nitroaldol addition.[21]

The straightforward preparation of **7** with complete stereocontrol from furfural, 2,2-dimethyl-1,3-dioxan-5-one, and 2,2,2-trimethoxyethyl benzoate in just 10 steps clearly showed the power of the new approach to assemble the dioxaadamantane core of tetrodotoxin.

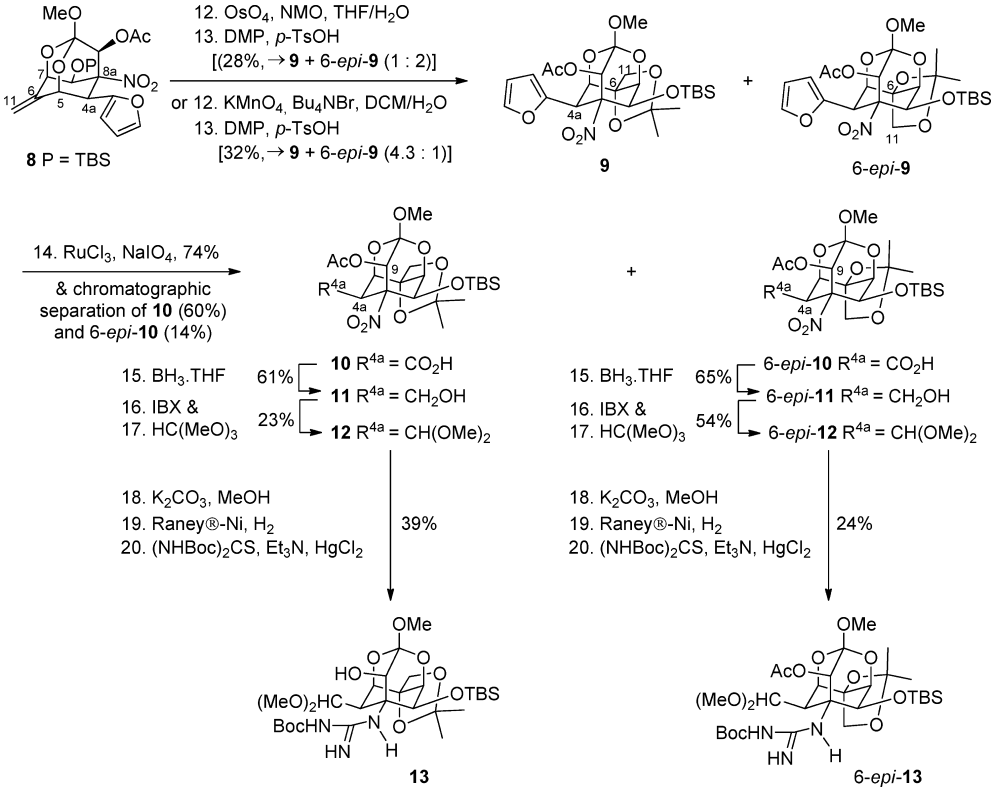
Installation of tetrodotoxin's full functionality in **7** required, in addition to any protection/deprotection steps, the dihydroxylation at C6 and C11, the transformation of the furan ring into a formyl group or equivalent, and the conversion of the nitro group into the guanidine functionality. In practice, we obtained better results when these three operations were carried out in the same order as listed above.

Thus, after protection of the free hydroxyl group of **7** in the form of acetate, as shown in **8**, the double bond was dihydroxylated, and the resulting diols were protected in the form of acetonides **9** and 6-*epi*-**9** (steps 12 and 13, Scheme 3). With OsO_4, the dihydroxylation preferentially took place from the less-hindered upper face of the alkene, giving double the amount of 6-*epi*-**9** compared with **9**. Alternative use of $KMnO_4$ reversed the facial selectivity in favor of **9** (**9**/6-*epi*-**9** ≈ 4/1), presumably because of repulsive interactions of the MnO_4^- anion with the oxygens at C5 and C7 in **8**.

The furan rings of **9** and 6-*epi*-**9** were then transformed into dimethyl acetal groups, as in **12** and 6-*epi*-**12**, through a four-step sequence (steps 14−17, Scheme 3). Treatment of the mixture of **9** and 6-*epi*-**9** with $RuCl_3/NaIO_4$ led to carboxylic acids **10** and 6-*epi*-**10**, which were separated by chromatog-

dx.doi.org/10.1021/jo500056w | J. Org. Chem. 2014, 79, 4300−4305

Scheme 3. Final Functionalization at C4a, C6, C8a, and C11



raphy and independently subjected to the successive action of BH_3·THF (to primary alcohols **11** and 6-*epi*-**11**, respectively), IBX (to the corresponding nonisolated aldehydes), and MeOH/HC(MeO)$_3$.

Finally, after deprotection of the acetate at C9 of **12**, the nitro group was reduced with Raney nickel, and the intermediate amine so formed was treated with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea, $HgCl_2$ and Et_3N to give **13**. Following a similar path, 6-*epi*-**12** was transformed into 6-*epi*-**13**. Protected guanidines **13** and 6-*epi*-**13** have every carbon atom required for tetrodotoxin and 6-*epi*-tetrodotoxin, respectively, with the correct relative configuration; the functionality at every position is also adequate: it either matches that of the toxin or its 6-*epi*-analogue or it is a protected form of it.

The successful preparation of **13** and 6-*epi*-**13** demonstrated that the functional groups present in **7** were appropriate to reach tetrodotoxin's full functionality, thus strengthening **7** as a promising advanced synthetic intermediate for TTX and analogues. However, its final transformation into the toxin itself and 6-*epi*-TTX through **13** and 6-*epi*-**13**, respectively, has been precluded so far by our failure to remove the methyl group from the orthoester function. The surprising stability of the methyl orthoester functionality was further corroborated in intermediate **7**, which either remained unchanged or gave complex mixtures when treated under a variety of acidic conditions. Further work (e.g., exploring the use of more labile protecting groups for the orthoester functionality) is then needed prior to the successful application of the route to the final target. In this respect, the convergent nature of the new synthetic sequence should be of help to prepare new dioxaadamantane intermediates similar to **7** but with improved functionality to facilitate their final conversion into tetrodotoxin and derivatives.

■ CONCLUSION

In summary, five commercially available fragments [furfural, 2-nitroethanol, 2,2-dimethyl-1,3-dioxan-5-one, chloroacetonitrile, and methyl(triphenyl)phosphonium bromide] and three main key steps [the (previously reported) formal (3 + 3) annulation of α-nitro-α,β-enals, a 1,3-diol orthoesterification, and an intramolecular Henry addition] are all combined in a relatively short (10 steps) and fully stereoselective pathway, to form advanced intermediate **7**, which displays the dioxaadamantane core of tetrodotoxin with the correct stereochemistry in all its newly formed seven stereogenic carbons and proved to be capable of being transformed into protected forms of TTX and 6-*epi*-TTX (**13** and 6-*epi*-**13**). Preliminary deprotection studies unveiled the difficulty to remove the orthoester's methyl group and the need for further experimentation before the new synthetic protocol could successfully render TTX.

■ EXPERIMENTAL SECTION

(1*R,3*R**,4*S**,5*R**,6*R**)-4-((*tert*-Butyldimethylsilyl)oxy)-6-(furan-2-yl)-2-methylene-5-nitrocyclohexane-1,3-diol (5).** To a solution of **3** (3 g, 10.1 mmol) in CH_2Cl_2 (20 mL) were added imidazole (6.869 g, 100.9 mmol) and *tert*-butyldimethylsilyl chloride (7.613 g, 50.5 mmol) at rt under argon. After being stirred for 24 h, the reaction mixture was diluted with a saturated aqueous solution of NH_4Cl (40 mL) and extracted with CH_2Cl_2 (3 × 50 mL). Chromatography (EtOAc/hexane 2:98) afforded the TBS ether of **3** (3.76 g, 9.14 mmol), which was dissolved in dry THF (40 mL) and added at −78 °C under argon to $Ph_3P{=}CH_2$ [itself prepared from $MePPh_3Br$ (13.06 g, 36.56 mmol) and *n*-BuLi (1.6 M in hexane, 20 mL, 32 mmol) in THF (40 mL)]. The reaction mixture was allowed to reach rt, stirred for 17 h, diluted with water (40 mL), and extracted with EtOAc (3 × 40 mL). After chromatography (EtOAc/hexane 3/

dx.doi.org/10.1021/jo500056w | J. Org. Chem. 2014, 79, 4300−4305

7), the intermediate alkene (3.2 g, 7.81 mmol, 87%) in dry THF (156 mL) was treated with a mixture of TFA/AcOH/H_2O (1:2:2, 156 mL) and stirred for 5 min. The pH of the mixture was adjusted to 8 with a saturated aqueous solution of $NaHCO_3$ and the solvent partially evaporated. Extraction (Et_2O, 3 × 300 mL) and chromatography (EtOAc/hexane 15/85) afforded (±)-**5** [1.71 g, 46%, R_f = 0.31 (EtOAc/hexane 3:7)] as an oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (td, J = 1.8, 0.7 Hz, 1H), 6.36 (td, J = 3.2, 0.7 Hz, 1H), 6.33 (dd, J = 3.2, 1.8 Hz, 1H), 5.41 (s, 1H), 5.38 (s, 1H), 5.28 (dd, J = 12.2, 9.6 Hz, 1H), 4.51 (dd, J = 7.2, 2.6 Hz, 1H), 4.41 (d, J = 3.1 Hz, 1H), 4.25 (dd, J = 9.6, 3.1 Hz, 1H), 3.50 (dd, J = 12.2, 2.6 Hz, 1H), 3.36 (d, J = 7.2 Hz, 1H), 3.18 (d, J = 1.3 Hz, 1H), 0.90 (s, 9H), 0.13 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.5, 142.3, 141.4, 120.4, 110.5, 108.3, 86.8, 76.3, 75.2, 74.0, 46.0, 25.5 (3 C), 17.8, −4.8, −5.5. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{17}H_{27}NNaO_6Si$ 392.1505; Found 392.1498.

((3*R,6*S**,7*R**,8*R**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(furan-2-yl)-3-methoxy-9-methylene-7-nitro-2,4-dioxabicyclo[3.3.1]-nonan-3-yl)methyl Benzoate (6a).** A mixture of (±)-**5** (0.615 g, 1.66 mmol), $(MeO)_3CCH_2OBz$ (6.05 g, 25.19 mmol), and *p*-TsOH·H_2O (0.635 g, 3.33 mmol) was heated to 100 °C. After being stirred for 4 h, the reaction mixture was diluted with a saturated aqueous solution of $NaHCO_3$ (50 mL), partially evaporated, and extracted with Et_2O (3 × 30 mL). Chromatography (EtOAc/hexane 15/85) afforded (±)-**6a** (0.612 g, 67%, R_f = 0.67) as an oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27−8.07 (m, 2H), 7.81−7.30 (m, 3H), 7.20 (dd, J = 1.8, 0.7 Hz, 1H), 6.18 (dd, J = 3.3, 1.8 Hz, 1H), 6.15 (d, J = 3.3 Hz, 1H), 5.51 (dd, J = 11.7, 9.2 Hz, 1H), 5.32 (s, 1H), 5.27 (s, 1H), 4.68 (t, J = 1.7 Hz, 1H), 4.59 (t, J = 1.9 Hz, 1H), 4.45 (d, J = 11.7 Hz, 1H), 4.35 (d, J = 11.7 Hz, 1H), 4.19 (dd, J = 9.2, 1.9 Hz, 1H), 3.48 (dd, J = 11.7, 1.7 Hz, 1H), 3.27 (s, 3H), 0.83 (s, 9H), 0.10 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 165.9, 148.9, 142.1, 138.2, 133.1, 129.8 (2 × C), 129.4, 128.4 (2xC), 111.6, 110.4, 110.1, 108.0, 89.2, 76.7, 75.7, 73.4, 63.3, 51.2, 46.3, 25.3 (3 × C), 17.7, −4.6, −5.5. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{27}H_{35}NNaO_9Si$ 568.1979; Found 568.1979.

(1*R,3*S**,5*R**,6*S**,7*S**,8*R**,10*S**)-6-((*tert*-Butyldimethylsilyl)-oxy)-8-(furan-2-yl)-3-methoxy-9-methylene-7-nitro-2,4-dioxaadamantan-10-ol (7).** Benzoate **6a** (0.472 g, 0.86 mmol) was stirred for 40 min in a solution of NaOMe in MeOH (6.3 mL, 3% w/v). After dilution with H_2O (50 mL) and extraction (CH_2Cl_2, 3 × 50 mL), the crude alcohol in dry CH_2Cl_2 (8 mL) was cooled to −78 °C and added to a mixture of oxalyl chloride (0.238 mL, 2.81 mmol) and DMSO (0.400 mL, 5.62 mmol) in dry CH_2Cl_2 (8 mL) at −78 °C under argon. The mixture was stirred for 30 min, treated with Et_3N (1.57 mL, 11.24 mmol), allowed to reach rt, diluted with H_2O (50 mL), and extracted with CH_2Cl_2 (3 × 40 mL). To a solution of the crude nitroaldehyde in dry THF (7 mL) were added NaH (0.043 g, 1.07 mmol) and small quantities of H_2O (≈500 μL) until the complete consumption of the starting material. Dilution with a saturated aqueous solution of NH_4Cl (50 mL), extraction (Et_2O, 2 × 30 mL), and chromatography (silica gel, EtOAc/hexane 2:8) afforded (±)-7 (0.160 g, 42%, R_f = 0.51) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (s, 1H), 6.33 (d, J = 3.4 Hz, 1H), 6.32 (d, J = 3.4 Hz, 1H), 5.25 (s, 1H), 5.20 (s, 1H), 5.08 (s, 1H), 4.81 (dd, J = 6.2, 0.6 Hz, 1H), 4.47 (t, J = 1.8 Hz, 1H), 4.40 (d, J = 1.8 Hz, 1H), 3.77 (s, 1H), 3.56 (s, 3H), 2.36 (d, J = 6.2 Hz, 1H), 0.91 (s, 9H), 0.13 (s, 1H), 0.07 (s, 3H). ^{13}C NMR and DEPT (125 MHz, $CDCl_3$) δ 149.3, 142.1, 138.4, 111.7, 110.6, 108.5, 92.1, 79.2, 77.2, 77.0, 76.9, 76.7, 75.7, 66.9, 50.1, 42.7, 25.4 (3 × C), 17.8, −4.7, −5.6. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{20}H_{29}NNaO_8Si$ 462.1560; Found 462.1563.

Acetate (±)-8. To a solution of compound 7 (1.46 g, 3.32 mmol) in CH_2Cl_2 (111 mL) were subsequently added Et_3N (3.7 mL, 26,6 mmol), Ac_2O (1.3 mL, 13,3 mmol), and DMAP (325 mg, 2.66 mmol) at rt under argon. After the mixture was stirred for 12 h, dilution with a saturated aqueous solution of NH_4Cl (50 mL), extraction (CH_2Cl_2, 3 × 50 mL), and chromatography (20% EtOAc/hexane) afforded acetate (±)-**8** (1.524 mg, 95%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 1.8 Hz, 1H), 6.30 (dd, J = 3.3, 1.8 Hz, 1H), 6.22 (td, J = 3.3, 1.0 Hz, 1H), 6.00 (d, J = 1.0 Hz, 1H), 5.22 (s, 1H), 5.17 (s, 1H), 5.08 (t, J = 1.2 Hz, 1H), 4.45 (t, J = 1.8 Hz, 1H), 4.42 (d, J = 1.8 Hz, 1H), 3.77 (bs, 1H), 3.44 (s, 3H), 1.67 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 149.4, 141.6, 138.1, 111.9, 110.3, 109.4, 107.9, 90.7, 79.1, 76.8, 75.4, 65.3, 50.2, 42.8, 25.3 (3 × C), 20.3, 17.8, −4.7, −5.6. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{22}H_{31}NNaO_9Si$ 504.1666; Found 504.1661.

Isopropylidene Acetals (±)-9 and (±)-6-*epi*-9. *a. Using $KMnO_4$.* To a solution of alkene (±)-**8** (1.288 g, 2.67 mmol) in CH_2Cl_2 (14.5 mL) at 0 °C were added *n*-Bu_4NBr (0.603 g, 1.87 mmol), H_2O (14,5 mL), and $KMnO_4$ (1.268 g, 8.02 mmol). The mixture was stirred for 45 min, allowed to warm until rt, concentrated, and filtered through silica gel (40% EtOAc/hexane). The crude diol in acetone (23 mL) was treated with 2,2-dimethoxypropane (DMP, 11 mL, 95.2 mmol) and *p*-TsOH·H_2O (0.406 g, 2.136 mmol). After the mixture was stirred for 15 min, Et_3N was added until basic pH, and the volatiles were removed in vacuo. Chromatography (silica gel, EtOAc/hexane 1:9) afforded a 4.3:1 mixture of **9** and 6-*epi*-**9** (0.468 g, 32% for the two steps) as an oil. Pure, small samples of each component could be obtained by careful column chromatography.

b. Using OsO_4. A 1:2 (36 mg, 28%) mixture of **9** and 6-*epi*-**9** was obtained by treating a solution of **8** (0.11 g, 0.23 mmol) in THF/H_2O (1:1, 3 mL) with NMO (475 mg, 4.06 mmol) and OsO_4 (1 M in H_2O, 54 mL, 0.054 mmol) for 18 h at rt, followed by workup (washing with saturated aqueous $Na_2S_2O_3$ (2 mL), evaporation of TFH, and extraction with EtOAc) and final protection of the crude diol as the acetonide as indicated in procedure a above.

9 + 6-*epi*-**9**: ^{13}C NMR (75 MHz, $CDCl_3$)168.5, 149.7, 141.7, 141.5, 111.5, 111.0, 110.3, 109.1, 108.8, 108.2, 107.8, 89.5, 89.4, 79.9, 78.8, 76.4, 76.3, 76.0, 75.1, 74.5, 73.2, 71.2, 67.8, 65.1, 53.3, 50.5, 49.9, 39.2, 37.8, 26.8, 26.6, 26.5, 25.3, 24.8, 20.2, 17.7, −4.7, −4.8, −5.6. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{25}H_{37}NNaO_{11}Si$ 578.2034; Found 578.2036.

(±)-**9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 1.8 Hz, 1H), 6.33 (dd, J = 3.3, 1.8 Hz, 1H), 6.20 (td, J = 3.3, 0.8 Hz, 1H), 5.94 (d, J = 0.8 Hz, 1H), 4.76 (d, J = 1.8 Hz, 1H), 4.58−4.46 (m, 1H), 4.39 (s, 2H), 4.21 (s, 1H), 3.95 (t, J = 1.8 Hz, 1H), 3.45 (s, 3H), 1.69 (s, 3H), 1.45 (s, 3H), 1.42 (s, 3H), 0.88 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H).

(±)-6-*epi*-**9**: ^{1}H MNR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 1.8 Hz, 1H), 6.33 (dd, J = 3.4, 1.8 Hz, 1H), 6.26 (td, J = 3.4, 0.8 Hz, 1H), 5.94 (s, 1H), 4.62 (dd, J = 2.2, 0.8 Hz, 1H), 4.26 (d, J = 1.5 Hz, 1H), 4.02 (t, J = 1.9 Hz, 1H), 3.96 (d, J = 9.5 Hz, 1H), 3.88 (d, J = 9.5 Hz, 1H), 3.60 (s, 1H), 3.55 (s, 3H), 1.69 (s, 3H), 1.52 (s, 3H), 1.50 (s, 3H), 0.88 (s, 9H), 0.11 (s, 3H), 0.07 (s, 3H).

Acids (±)-10 and (±)-6-*epi*-10. A solution of a mixture of (±)-**9** and (±)-6-*epi*-**9** (4.3:1, 285 mg, 0.51 mmol) in $CH_3CN/CCl_4/H_2O$ (3/3/4 mL) was treated with $NaIO_4$ (0.872 mg, 4.08 mmol) and $RuCl_3$ (0.034 mg, 0.15 mmol). After the mixture was stirred for 2 h, silica gel (5 g) was added and the mixture concentrated. Chromatography (silica gel, 1% AcOH, 40% EtOAc/hexane) afforded **10** (164 mg, 60%) and 6-*epi*-**10** (38 mg, 14%) as oils.

10 + 6-*epi*-**10**: HRMS (ESI-TOF) m/z: (M + Na)$^+$ Calcd for $C_{22}H_{35}NNaO_{12}Si$ 556.1826; Found 556.1821. (±)-**10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (s, 1H), 4.63 (s, 1H), 4.63 (s, 1H), 4.31 (s, 2H), 3.88 (t, J = 1.6 Hz, 1H), 3.74 (s, 1H), 3.38 (s, 3H), 1.97 (s, 3H), 1.41 (s, 6H), 0.85 (s, 9H), 0.05 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 168.2, 111.7, 108.9, 86.9, 78.4, 74.4, 74.4, 73.2, 71.0, 65.4, 49.9, 43.0, 26.6, 26.5, 25.3 (3 × C), 20.5, 17.8, −4.8, −5.6.

(±)-6-*epi*-**10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (s, 1H), 4.72 (s, 1H), 4.14 (s, 1H), 3.96 (s, 1H), 3.90 (d, J = 9.5 Hz, 1H), 3.81 (d, J = 9.5 Hz, 1H), 3.50 (s, 3H), 3.16 (s, 1H), 2.01 (s, 3H), 1.51 (s, 3H), 1.48 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 168.3, 111.2, 108.7, 87.1, 79.7, 75.7, 74.6, 74.5, 67.6, 65.4, 50 0.5, 44.0, 26.8, 26.8, 25.3 (3 × C), 20.6, 17.8, −4.7, −5.6.

(1*R,3*S**,4′*S**,5*S**,6*S**,7*S**,8*S**,10*S**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(hydroxymethyl)-3-methoxy-2′,2′-dimethyl-7-nitro-2,4-dioxaspiro[adamantane-9,4′-[1,3]dioxolan]-10-yl Acetate (11).** To a solution of (±)-**10** (106 mg, 0.199 mmol) in dry THF (2 mL) at rt under argon were added BH_3·THF (1 M in THF, 0.795 mL, 0.795 mmol) and $NaBH_4$ (8 mg, 0.199 mmol). After being stirred for 14 h at rt, the reaction mixture was diluted with a saturated

dx.doi.org/10.1021/jo500056w | J. Org. Chem. 2014, 79, 4300−4305

aqueous solution of NH_4Cl (50 mL) until pH 7 and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried and concentrated in vacuo. Chromatography (40% EtOAc/hexane) afforded **11** (63 mg, 61%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (d, J = 0.8 Hz, 1H), 4.57 (d, J = 1.7 Hz, 1H), 4.32 (s, 2H), 4.27 (bs, 1H), 4.10 (dd, J = 10.7, 9.8 Hz, 1H), 3.88 (t, J = 1.8 Hz, 1H), 3.53 (dd, J = 11.1, 3.2 Hz, 1H), 3.43 (s, 1H), 3.40 (s, 3H), 2.94 (dd, J = 9.3, 3.0 Hz, 1H), 2.05 (s, 3H), 1.42 (s, 3H), 1.41 (s, 3H), 0.85 (s, 9H), 0.04 (s, 3H), −0.02 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 111.4, 109.0, 98.8, 88.9, 79.3, 74.9, 73.7, 71.1, 65.6, 58.8, 49.9, 42.7, 26.7, 26.5, 25.4(3 × C), 20.8, 17.8, −4.9, −5.7. Calcd for $C_{22}H_{37}NNaO_{11}Si$ 542.2034; Found 542.2036.

(1*R,3*S**,4′*R**,5*S**,6*S**,7*S**,8*S**,10*S**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(hydroxymethyl)-3-methoxy-2′,2′-dimethyl-7-nitro-2,4-dioxaspiro[adamantane-9,4′-[1,3]dioxolan]-10-yl Acetate (6-*epi*-11).** 6-*epi*-**11** was obtained (chromatography (40% EtOAc/hexane), oil, 67 mg, 65%) from (±)-6-*epi*-**10** (106 mg, 0,199 mmol), BH_3·THF (1 M in THF, 0.795 mL, 0.795 mmol) and $NaBH_4$ (8 mg, 0.199 mmol), as described for (±)-**11**. ^{1}H NMR (500 MHz $CDCl_3$) δ 6.00 (s, 1H), 4.36 (d, J = 1.7 Hz, 1H), 4.21 (dd, J = 10.5, 9.5 Hz, 1H), 4.15 (bs, 1H), 4.00 (t, J = 2.0 Hz, 1H), 3.93 (d, J = 9.5 Hz, 1H), 3.87 (d, J = 9.4 Hz, 1H), 3.61−3.50 (m, 4H), 2.35 (dd, J = 9.1, 2.6 Hz, 1H), 2.10 (s, 3H), 1.52 (s, 6H), 0.90 (s, 9H), 0.11 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.3, 110.8, 108.7, 88.8, 80.4, 76.4, 75.4, 75.0, 67.8, 65.5, 58.6, 50.5, 44.3, 26.9, 26.6, 25.3, 20.8, 17.8, −4.7, −5.6. HRMS (ESI-TOF) m/z: $(M + Na)^+$ Calcd for $C_{22}H_{37}NNaO_{11}Si$ 542.2034; Found 542.2036.

(1*R,3*S**,4′*S**,5*S**,6*S**,7*S**,8*R**,10*S**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(dimethoxymethyl)-3-methoxy-2′,2′-dimethyl-7-nitro-2,4-dioxaspiro[adamantane-9,4′-[1,3]dioxolan]-10-yl Acetate (12).** To a solution of nitroalcohol **11** (0.120 g, 0.23 mmol) in EtOAc was added IBX (0.192 g, 0.69 mmol). After being stirred for 8 h at 80 °C, the reaction mixture was cooled to 0 °C and filtered. Rotary evaporation rendered the crude aldehyde, which was dissolved in a mixture of dry THF:MeOH (1:1, 6 mL) and stirred with p-TsOH (0.001 g, 0.0023 mmol) and trimethoxymethane (0.049 g, 0.46 mmol) for 24 h under argon. The pH was adjusted to 7 with Et_3N. Adsorption on SiO_2 (0.1 g) followed by chromatography (EtOAc/hexane, 2:8) gave (±)-**12** (0.03 g, 23%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.03 (s, 1H), 5.14 (d, J = 9.0 Hz, 1H), 4.65 (d, J = 1.6 Hz, 1H), 4.33 (d, J = 9.5 Hz, 1H), 4.28 (d, J = 9.5 Hz, 1H), 4.18 (s, 1H), 3.87 (t, J = 1.6 Hz, 1H), 3.42 (s, 3H), 3.31 (s, 3H), 3.30 (s, 3H), 3.09 (d, J = 9.0 Hz, 1H), 2.15 (s, 3H), 1.44 (s, 3H), 1.43 (s, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 111.4, 109.0, 101.0, 86.7, 79.0, 75.4, 74.9, 74.1, 71.2, 66.1, 55.8, 50.4, 50.0, 41.3, 26.7, 26.6, 25.4 (3 × C), 21.2, 17.8, −4.8, −5.7. HRMS (ESI-TOF) m/z: $(M + Na)^+$ Calcd for $C_{24}H_{41}NNaO_{12}Si$ 586.2296; Found 586.2297.

(1*R,3*S**,4′*R**,5*S**,6*S**,7*S**,8*R**,10*S**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(dimethoxymethyl)-3-methoxy-2′,2′-dimethyl-7-nitro-2,4-dioxaspiro[adamantane-9,4′-[1,3]dioxolan]-10-yl Acetate (6-*epi*-12).** 6-*epi*-**12** was obtained (chromatography (EtOAc/hexane 2/8), oil, 70 mg, 54%) from 6-*epi*-**11** (0.120 g, 0.23 mmol), IBX (0.192 g, 0.69 mmol), p-TsOH (0.001 g, 0.0023 mmol), and trimethoxymethane (0.049 g, 0.46 mmol), as described for (±)-**12**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.03 (d, J = 1.1 Hz, 1H), 5.11 (d, J = 8.5 Hz, 1H), 4.22 (dd, J = 2.2, 0.8 Hz, 1H), 4.14 (d, J = 1.6 Hz, 1H), 3.94 (dd, J = 2.2, 1.6 Hz, 1H), 3.87−3.80 (m, 2H), 3.52 (s, 3H), 3.42 (s, 3H), 3.32 (s, 3H), 2.38 (td, J = 8.5, 1.0, 1.0 Hz, 1H), 2.16 (s, 3H), 1.58 (s, 3H), 1.48 (s, 3H), 1.48 (s, 3H), 0.86 (s, 9H), 0.08 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 110.9, 108.7, 102.2, 86.7, 79.9, 76.1, 76.0, 75.2, 67.9, 66.0, 56.4, 54.0, 50.6, 44.1, 26.9, 26.7, 25.3 (3 × C), 21.2, 17.8, −4.6, −5.7. HRMS (ESI-TOF) m/z: $(M + Na)^+$ Calcd for $C_{24}H_{41}NNaO_{12}Si$ 586.2296; Found 586.2297.

Guanidine (±)-13. A solution of (±)-**12** (25 mg, 0.044 mmol) in K_2CO_3-saturated MeOH (1 mL) was stirred for 4 h at rt. The mixture was diluted with a saturated aqueous solution of NH_4Cl until pH 6, partially evaporated, and extracted (CH_2Cl_2, 3 × 5 mL). Chromatography (30% EtOAc/hexane) afforded the corresponding alcohol intermediate (11 mg), a portion of which (4 mg) together with Raney nickel (≈7 mL) in MeOH (1 mL) was stirred at rt under H_2 until complete reduction (as monitored by TLC). The catalyst was filtered and washed with MeOH (5 mL) and EtOAc (5 mL). The crude amine obtained by solvent removal was dissolved in dry DMF (1 mL) and stirred with $(NHBoc)_2CS$ (2.4 mg, 0.008 mmol), $HgCl_2$ (2.3 mg, 0.008 mmol), and Et_3N (2 μL, 0.012 mmol) for 1 h. Filtration over Celite, washing with $CHCl_3$, solvent removal, and chromatography (EtOAc/hexane, 2:8) afforded (±)-**13** (3 mg, 39%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 6.63 (s, 1H), 4.80 (d, J = 1.8 Hz, 1H), 4.65 (d, J = 3.8 Hz, 1H), 4.33 (d, J = 9.2 Hz, 1H), 4.26 (d, J = 2.2 Hz, 1H), 4.22 (d, J = 9.2 Hz, 1H), 4.14 (d, J = 9.8 Hz, 1H), 3.93 (d, J = 9.8 Hz, 1H), 3.76 (dd, J = 2.2, 1.8 Hz, 1H), 3.42 (s, 3H), 3.39 (s, 3H), 3.33 (s, 3H), 3.15 (d, J = 3.8 Hz, 1H), 1.48 (s, 9H), 1.36 (s, 6H), 0.86 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 151.4, 110.8, 109.0, 103.9, 82.9, 79.9, 76.0, 74.1, 71.4, 70.0, 67.4, 59.9, 55.8, 54.6, 49.2, 38.2, 27.9 (3 × C), 26.7, 26.7, 25.6 (3 × C), 17.9, −4.7, −5.2. HRMS (ESI-TOF) m/z: $(M + Na - CH_3OH)^+$ Calcd for $C_{27}H_{48}N_3NaO_{10}Si$ 625.3007; Found 625.3012.

Guanidine (±)-6-*epi*-13. 6-*epi*-**13** was obtained (chromatography (EtOAc/hexane 2/8), oil, 8 mg, 24%) from 6-*epi*-**12** (30 mg, 0.053 mmol) as described for (±)-**13**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 6.53 (s, 1H), 5.14 (s, 1H), 4.95 (d, J = 2.0 Hz, 1H), 4.29 (d, J = 9.1 Hz, 1H), 4.21 (d, J = 9.1 Hz, 1H), 4.15 (s, 1H), 3.84 (t, J = 2.0 Hz, 1H), 3.50 (d, J = 3.7 Hz, 1H), 3.43 (s, 3H), 3.35 (s, 3H), 2.97 (s, 1H), 1.49 (s, 9H), 1.41 (s, 3H), 1.37 (s, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). HRMS (ESI-TOF) m/z: $(M + Na + 1)^+$ Calcd for $C_{28}H_{52}N_3NaO_{11}Si^+$ 657.3269; Found 657.3272.

DFT Calculations. DFT calculations were carried out using the Gaussian 09 software package[22] and the model chemistry M06-L/6-31G(d,p) (i.e., the combination of the local version of the Minnesota 2006 meta GGA density functional, M06-L,[23] developed by Truhlar and co-workers, with Pople's double-ζ basis set, 6-31G(d,p),[24] which includes polarization functions on all atoms). All stationary points, i.e., stable structures and transitions states, were optimized, and normal-mode analyses were performed to verify that the stable structures have all positive frequencies and that the transition states have only one imaginary frequency each with the corresponding eigenvector pointing toward the reactant or product. All transition structures were located using the Berny algorithm; calculations of the minimum energy path (MEP)[25] confirmed that the located transition structures connected reactants and products. Thermodynamic functions were computed using the rigid-rotor and harmonic oscillator approximation.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for **5**−**13** and 6-*epi*-**9**−**13** and electronic structure calculations. Coordinates along with thermochemical data for each calculated structure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support by the Ministry of Science and Innovation (Project CTQ2008-03253) and by the Xunta de Galicia (Projects 05BTF20901PR, 08CSA046209PR, 2007/XA084, and CN2011/054) is gratefully acknowledged. H.L.S. acknowledges contracts with financial support from the Xunta de Galicia. We thank the Center of Supercomputing of Galicia

(CESGA, https://www.cesga.es/en/cesga) for the computational facilities.

DEDICATION

§This paper is dedicated, with appreciation and gratitude, to the Professors of Organic Chemistry Domingo Domínguez, Enrique Guitián, and Antonio Mouriño.

REFERENCES

(1) (a) *Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel*; Kao, C. Y., Levinson, S. R., Eds.; New York Academy of Sciences: New York, 1986; Vol. *479*. (b) Moczydlowski, E. G. The molecular mystique of tetrodotoxin. *Toxicon* **2013**, *63*, 165−183.

(2) Noda, M.; Shimizu, S.; Tanabe, T.; Takai, T.; Kayano, T.; Ikeda, T.; Takahashi, H.; Nakayama, H.; Kanaoka, Y.; Minamino, N.; Kangawa, K.; Matsuo, H.; Raftery, M. A.; Hirose, T.; Inayama, S.; Hayashida, H.; Miyata, T.; Numa, S. *Nature* **1984**, *312*, 121−127.

(3) See for example: (a) Walker, J. R.; Novick, P. A.; Parsons, W. H.; McGregor, M.; Zablocki, J.; Pande, V. S.; Du Bois, J. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 18102−18107. (b) Huang, C.-J.; Schild, L.; Moczydlowski, E. G. *J. Gen. Physiol.* **2012**, *140*, 435−454.

(4) Mosher, H. S. The chemistry of tetrodotoxin. *Ann. N.Y. Acad. Sci.* **1986**, *479*, 32−43.

(5) Tetrodotoxin is the main lead compound in the drug development program of the company WexPharmaceuticals: http://www.wexpharma.com/.

(6) The structural determination of tetrodotoxin was independently reported at the third IUPAC Symposium on the Chemistry of Natural Products, 13 April, 1964 (Kyoto, Japan), by four research groups: (a) Goto, T.; Kishi, Y.; Takahashi, S.; Hirata, Y. *Tetrahedron* **1965**, *21*, 2059−2088. (b) Mosher, H. S.; Fuhrman, F. A.; Buchwald, H. D.; Fischer, H. G. *Science* **1964**, *144*, 1100−1110. (c) Tsuda, K.; Ikuma, S.; Kawamura, M.; Tachikawa, R.; Sakai, K.; Tamura, C.; Amakasu, O. *Chem. Pharm. Bull.* **1964**, *12*, 1357−1374. (d) Woodward, R. B. *Pure Appl. Chem.* **1964**, *9*, 49−74.

(7) (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*, 9217−9219. (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*, 9219−9221.

(8) More recently, the group led by Sato reported a stereocontrolled synthesis of racemic tetrodotoxin from *myo*-inositol: Sato, K.-i.; Akai, S.; Sugita, N.; Ohsawa, T.; Kogure, T.; Shoji, H.; Yoshimura, J. *J. Org. Chem.* **2005**, *70*, 7496−7504.

(9) For asymmetric total syntheses of (−)-tetrodotoxin, see: (a) Ohyabu, N.; Nishikawa, T.; Isobe, M. *J. Am. Chem. Soc.* **2003**, *125*, 8798−8805. (b) Hinman, A.; Du Bois, J. *J. Am. Chem. Soc.* **2003**, *125*, 11510−11511. (c) Nishikawa, T.; Urabe, D.; Isobe, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4782−4785. (d) Noheda, P. Synthesis of tetrodotoxin, its analogues and intermediates thereof. EP 1785427, 2007. (e) Sato, K.-i.; Akai, S.; Shoji, H.; Sugita, N.; Yoshida, S.; Nagai, Y.; Suzuki, K.; Nakamura, Y.; Kajihara, Y.; Funabashi, M.; Yoshimura, J. *J. Org. Chem.* **2008**, *73*, 1234−1242. (f) Akai, S.; Seki, H.; Sugita, N.; Kogure, T.; Nishizawa, N.; Suzuki, K.; Nakamura, Y.; Kajihara, Y.; Yoshimura, J.; Sato, K. *Bull. Chem. Soc. Jpn.* **2010**, *83*, 279.

(10) In his highlight "Syntheses of Tetrodotoxin" (Koert, U. *Angew. Chem., Int. Ed.* **2004**, *43*, 5572−5576), and referring to the syntheses of Isobe and Du Bois, refs 9a−c above, the author concludes: "All three syntheses are long (> 25 steps) and follow a linear strategy. The hexasubstituted cyclohexane intermediate makes a convergent strategy, which may reduce the number of steps to less than 20, very difficult but not impossible. Because of its structural complexity and functional-group density, tetrodotoxin will remain a prime synthetic target".

(11) For a recent review on the synthesis of tetrodotoxin, see: Chau, J.; Ciufolini, M. A. *Mar. Drugs* **2011**, *9*, 2046−2074. For recent synthetic work, see, for example: Adachi, M.; Imazu, T.; Isobe, M.; Nishikawa, T. *J. Org. Chem.* **2013**, *78*, 1699−1705.

(12) Cagide-Fagín, F.; Alonso, R. *Eur. J. Org. Chem.* **2010**, *2010*, 6741−6747.

(13) Martínez-Bescos, P.; Cagide-Fagín, F.; Roa, L. F.; Ortiz-Lara, J. C.; Kierus, K.; Ozores-Viturro, L.; Fernández-González, M.; Alonso, R. *J. Org. Chem.* **2008**, *73*, 3745−3753.

(14) (a) Ortiz, J. C.; Ozores, L.; Cagide-Fagin, F.; Alonso, R. *Chem. Commun.* **2006**, 4239−4241. (b) Cagide-Fagín, F.; Nieto-García, O.; Lago-Santomé, H.; Alonso, R. *J. Org. Chem.* **2012**, *77*, 11377−11382. (c) For a review on the enantioselective organocatalyzed domino synthesis of six-membered carbocycles, see: Goudedranche, S.; Raimondi, W.; Bugaut, X.; Constantieux, T.; Bonne, D.; Rodriguez, J. *Synthesis* **2013**, *45*, 1909−1930.

(15) Except for IUPAC names in the Experimental Section, the numbering of tetrodotoxin is used in this article.

(16) (a) Hugel, H. M.; Bhaskai, K. V.; Longmore, R. W. *Synth. Commun.* **1992**, *22*, 693. (b) Endová, M.; Masojidková, M.; Budějínský, M.; Rosenberg, I. *Tetrahedron* **1998**, *54*, 11187. (c) McElvain, S. M.; Nelson, J. W. *J. Am. Chem. Soc.* **1942**, *64*, 1825.

(17) Orthoesters **6** were assumed to preferentially adopt the conformation shown in Scheme 2 by analogy with the structurally related acetal **3**, which exclusively exists in such a conformation in the solid state (see ref 14a).

(18) Antiperiplanar arrangement of one lone pair of each oxygen atom of their 1,3-dioxane ring with the exocyclic C−O bond. See, for example, Kirby, A. J. *The anomeric effect and related stereoelectronic effects at oxygen*; Springer-Verlag: Duesseldorf, 1983; pp 21−22.

(19) These two factors should increase comparatively with acetal **3**, the preference of orthoesters **6** for the conformation shown in Scheme 2.

(20) See the Supporting Information.

(21) While hydrogen bonding would certainly stabilize the reacting conformation **A** shown in Scheme 2, the anion intermediate (as well as its precursor, the orthoester **6c**) could enjoy the conformational equilibrium that the 1,3-dioxane unit of compound **3** appears to have in solution ($CDCl_3$) between a most stable "boatlike" conformation (the only one present in the solid form; see ref 18) and a "chair-type" conformation, where one of the methyl groups of the acetal bridge is close enough to H8a to cause (when saturated during ^{1}H NMR acquisition) a weak (1%) NOE enhancement of its ^{1}H NMR signal.

(22) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A.02*; Gaussian, Inc., Wallingford, CT, 2009.

(23) Zhao, Y.; Truhlar, D. G. *J. Chem. Phys.* **2006**, *125*, 194101−18.

(24) (a) Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724−728. (b) Hariharan, P. C.; Pople, J. A. *Chem. Phys. Lett.* **1972**, *66*, 217−219.

(25) Truhlar, D. G.; Kuppermann, A. *J. Am. Chem. Soc.* **1971**, *93*, 1840−1851.