

# **A Stereoselective Synthesis of (-)-Tetrodotoxin**

## **Supplementary Material**

*(8 pages)*

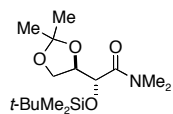
Andrew Hinman and J. DuBois

*Department of Chemistry  
Stanford University  
Stanford, CA 94305-5080*

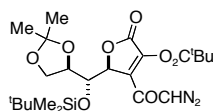
**General.** All reagents were obtained commercially unless otherwise noted. Reactions were performed using flame-dried glassware under an atmosphere of nitrogen. Air and moisture sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Organic solutions were concentrated under reduced pressure (ca. 15 mm Hg) by rotary evaporation. Dichloromethane, dichloroethane, triethylamine, pyridine, and methanol were freshly distilled from CaH<sub>2</sub> immediately prior to use. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl immediately prior to use. Chlorobenzene was used as purchased. *N,N*-Dimethylformamide was allowed to stand over 4 Å molecular sieves prior to use. Air and moisture sensitive solids were weighed and transferred in an inert atmosphere N<sub>2</sub> glovebox. Rhodium on carbon was activated by heating at 55 °C under vacuum (ca. 1 mm Hg) for 3 hr. Chromatographic purification of products was accomplished using forced-flow chromatography on EM Science Geduran silica gel 60 (35–75 μm). Thin layer chromatography (TLC) was performed on EM Science silica gel 60 F<sub>254</sub> plates (250 μm). Visualization of the developed chromatogram was accomplished by fluorescence quenching and by staining with ethanolic anisaldehyde or aqueous ceric ammonium molybdate (CAM) solution. High performance liquid chromatography (HPLC) was performed on a Beckman 125S or a Rainin instrument using a Phenomenex Spherisorb 5-CN column (4.6 x 150 mm). The products were eluted with a 1% MeOH/99% 5 mM HCO<sub>2</sub>NH<sub>4</sub>/HCO<sub>2</sub>H buffer (pH 8.2) and were detected at 205 nm. A sample of natural (–)-tetrodotoxin was obtained from CalBioChem (San Diego, CA).

Nuclear magnetic resonance (NMR) spectra were acquired on a Varian Inova spectrometer operating at 500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively, and are referenced internally to solvent signals. Data for <sup>1</sup>H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; br s, broad singlet; d, doublet; t, triplet; m, multiplet), integration, coupling constant (Hz). Data for <sup>13</sup>C NMR are recorded in terms of chemical shift (δ, ppm). Infrared spectra were collected on a Thermo Nicolet IR300 spectrometer and are reported in terms of frequency of absorption. Sample preparation was done as a thin film on a NaCl plate or as a KBr pellet. Optical rotation data were obtained from samples loaded into a 50 mm cell on a Jasco DIP-1000 digital polarimeter operating at the Na D-line. High-resolution mass spectra were obtained from the Mass Spectrometry Facility, University of California and San Francisco, supported by the NIH Division of Research and Resources.

### Characterization data for select compounds

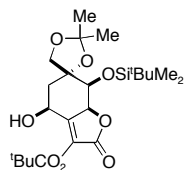


***N,N*-Dimethylamide 3:** TLC  $R_f = 0.57$  (1:1 hexanes/EtOAc);  $[\alpha]_{Na} +21.5^\circ$  ( $c = 3.00$  in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 4.44 (d, 1H,  $J = 7.2$  Hz), 4.28 (dd, 1H,  $J = 6.5, 6.2$  Hz), 4.08 (dd, 1H,  $J = 8.6, 6.3$  Hz), 3.99 (dd, 1H,  $J = 8.5, 5.6$  Hz), 3.14 (s, 3H), 2.97 (s, 3H), 1.43 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H) ppm; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 170.6, 109.6, 76.9, 72.4, 66.6, 36.9, 36.1, 26.4, 25.6, 25.0, 18.1, –4.8, –5.3 ppm; IR (thin film)  $\nu$  2930, 2858, 1657, 1255, 1157, 1104, 1072, 837, 779 cm<sup>–1</sup>. HRMS (EI) calcd for C<sub>15</sub>H<sub>31</sub>NO<sub>4</sub>Si 317.2022 found 302.1792 (M<sup>+</sup>–CH<sub>3</sub>).

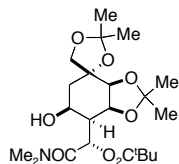


**Diazoketone 8:** TLC  $R_f = 0.33$  (4:1 hexanes/EtOAc);  $[\alpha]_{Na} +138^\circ$  ( $c = 0.10$  in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.75 (s, 1H), 5.43 (s, 1H), 4.29 (br d, 1H,  $J = 6.9$  Hz), 4.02–3.95 (m, 2H), 3.85 (dd, 1H,  $J = 8.2, 5.2$  Hz), 1.39 (s,

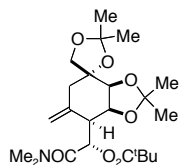
9H), 1.31 (s, 3H), 1.27 (s, 3H), 0.90 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  178.2, 173.7, 165.7, 140.3, 139.4, 109.5, 81.6, 73.1, 66.8, 58.1, 39.3, 26.9, 26.3, 25.6, 25.2, 17.9, -4.5, -4.8 ppm; IR (thin film)  $\nu$  2934, 2115, 1786, 1674, 1610, 1404, 1372, 1320, 1258, 1120, 1072, 839, 779  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{23}\text{H}_{36}\text{N}_2\text{O}_8\text{Si}$  496.2241 found 481.1993 ( $\text{M}^+-\text{CH}_3$ ).



**Butenolide:** TLC  $R_f$  = 0.22 (4:1 hexanes/EtOAc);  $[\alpha]_{\text{Na}} +31.0^\circ$  ( $c$  = 0.10 in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 500 MHz)  $\delta$  4.66 (dd, 1H,  $J$  = 3.4, 0.5 Hz), 4.51 (ddd, 1H,  $J$  = 11.5, 6.8, 6.2 Hz), 3.92 (d, 1H,  $J$  = 3.8 Hz), 3.90 (d, 1H,  $J$  = 9.0 Hz), 3.38 (d, 1H,  $J$  = 9.0 Hz), 1.96 (dd, 1H,  $J$  = 12.2, 11.5 Hz), 1.89 (d, 1H,  $J$  = 6.8 Hz), 1.76 (dd, 1H,  $J$  = 12.3, 6.6 Hz), 1.21 (s, 9H), 1.17 (s, 3H), 1.16 (s, 3H), 0.85 (s, 9H), 0.28 (s, 3H), -0.07 (s, 3H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  176.6, 167.0, 148.3, 131.5, 110.9, 82.0, 77.1, 72.3, 71.5, 66.1, 39.2, 39.0, 27.2, 26.9, 26.6, 25.6, 17.9, -4.4, -5.0 ppm; IR (thin film)  $\nu$  3500, 2959, 1770, 1699, 1635, 1481, 1372, 1257, 1216, 1139, 1092, 874, 839, 828, 781  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{23}\text{H}_{38}\text{O}_8\text{Si}$  470.2336 found 455.2101 ( $\text{M}^+-\text{CH}_3$ ).

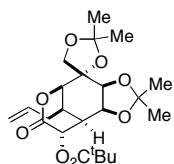


**Secondary Alcohol:** TLC  $R_f$  = 0.28 (4:1  $\text{CH}_2\text{Cl}_2$ /EtOAc);  $[\alpha]_{\text{Na}} +75.0^\circ$  ( $c$  = 0.10 in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.62 (d, 1H,  $J$  = 10.6 Hz), 4.73 (d, 1H,  $J$  = 7.6 Hz), 4.16 (dd, 1H,  $J$  = 7.6, 1.7 Hz), 4.14 (d, 1H,  $J$  = 9.1 Hz), 3.91–3.85 (m, 1H), 3.65 (d, 1H,  $J$  = 9.2 Hz), 3.30 (s, 3H), 2.99 (s, 3H), 2.86 (ddd, 1H,  $J$  = 10.6, 4.5, 2.0 Hz), 2.71 (d, 1H,  $J$  = 11.7 Hz), 2.37 (ddd, 1H,  $J$  = 15.1, 9.6, 1.7 Hz), 1.82 (dd, 1H,  $J$  = 15.1, 4.4 Hz), 1.48 (s, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.29 (s, 3H), 1.24 (s, 9H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  178.1, 169.2, 110.7, 108.9, 78.9, 77.1, 73.2, 71.6, 68.4, 65.2, 40.0, 39.2, 38.7, 37.6, 36.2, 27.1, 27.0, 26.9, 26.6, 23.0 ppm; IR (thin film)  $\nu$  3437, 2981, 2934, 1729, 1643, 1479, 1381, 1282, 1259, 1213, 1152, 1064, 978, 884  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{22}\text{H}_{37}\text{NO}_8$  443.2519 found 428.2312 ( $\text{M}^+-\text{CH}_3$ ).



**Olefin 11:** TLC  $R_f$  = 0.46 (11:2  $\text{CH}_2\text{Cl}_2$ /EtOAc);  $[\alpha]_{\text{Na}} -114^\circ$  ( $c$  = 1.00 in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 500 MHz)  $\delta$  6.01 (d, 1H,  $J$  = 8.7 Hz), 4.94 (dd, 1H,  $J$  = 7.3, 3.2 Hz), 4.92 (d, 1H,  $J$  = 1.7 Hz), 4.82 (d, 1H,  $J$  = 1.8 Hz), 4.32 (dd, 1H,  $J$  = 7.2, 1.4 Hz), 4.26 (d, 1H,  $J$  = 9.0 Hz), 4.01 (br d, 1H,  $J$  = 7.7 Hz), 3.59 (d, 1H,  $J$  = 9.0 Hz), 2.85 (s, 3H), 2.65 (dd, 1H,  $J$  = 16.2, 1.1 Hz), 2.59 (s, 3H), 2.43 (d, 1H,  $J$  = 16.2 Hz), 1.36 (s, 3H), 1.29 (s, 3H), 1.21 (s, 3H), 1.20 (s, 9H), 1.18 (s, 3H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  177.5, 169.0, 140.3, 110.7, 110.4, 108.8, 79.9, 77.4, 73.4, 71.7, 68.0, 42.1, 38.8, 38.4, 37.4, 36.1, 27.2, 27.0, 26.7, 26.3, 24.4 ppm; IR (thin film)  $\nu$  2984, 2937, 2873, 1732, 1659,

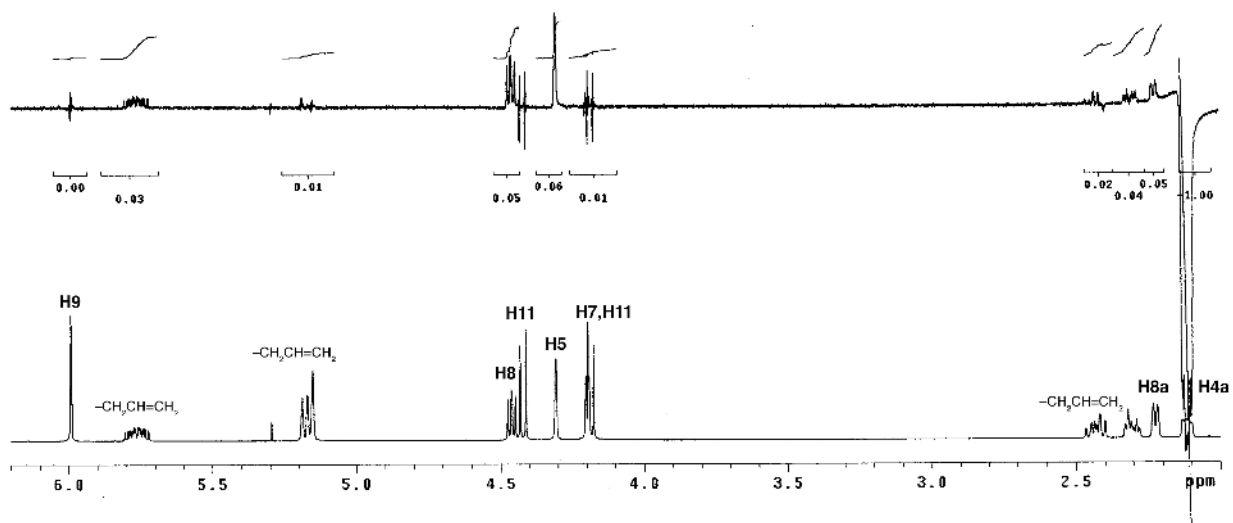
1480, 1381, 1371, 1258, 1211, 1157, 1064, 1033, 938, 881, 829  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{23}\text{H}_{37}\text{NO}_7$  439.2570 found 424.2329 ( $\text{M}^+ - \text{CH}_3$ ).



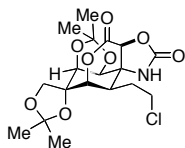
**Lactone 14:** TLC  $R_f = 0.65$  (2:1 hexanes/EtOAc);  $[\alpha]_{\text{Na}} -93.3^\circ$  ( $c = 0.50$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  5.99 (s, 1H), 5.80–5.72 (m, 1H), 5.19–5.15 (m, 2H), 4.46 (dd, 1H,  $J = 7.2, 6.0$  Hz), 4.42 (d, 1H,  $J = 9.9$  Hz), 4.31 (d, 1H,  $J = 1.1$  Hz), 4.20–4.17 (m, 2H), 2.46–2.40 (m, 1H), 2.32–2.28 (m, 1H), 2.22 (br d, 1H,  $J = 7.2$  Hz), 2.13–2.10 (m, 1H), 1.63 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H), 1.37 (s, 3H), 1.21 (s, 9H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  176.3, 166.1, 134.7, 118.2, 111.2, 109.6, 79.4, 78.9, 77.7, 73.4, 68.9, 64.6, 40.2, 38.5, 34.2, 29.8, 27.1, 27.0, 26.0, 25.1, 25.0 ppm; IR (thin film)  $\nu$  2985, 2935, 1752, 1742, 1383, 1373, 1253, 1210, 1156, 1134, 1060, 910, 852, 838, 734  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{23}\text{H}_{34}\text{O}_8$  438.2254 found 423.2032 ( $\text{M}^+ - \text{CH}_3$ ).

**Select coupling constant and nOe data for Lactone 14:**

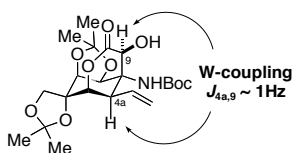
	<i>coupling constants</i>	<i>nOe data</i>
	$J_{4a,5}$ 1.1 Hz	$\text{H}_{4a} \rightarrow \text{H}_8$ : 5%
	$J_{4a,8a}$ $\leq 1$ Hz	$\text{H}_{4a} \rightarrow \text{H}_5$ : 6%
	$J_{8a,8}$ 7.2 Hz	$\text{H}_{4a} \rightarrow \text{H}_{8a}$ : 5%
	$J_{8a,9}$ 0 Hz	
	$J_{8,7}$ 6.0 Hz	



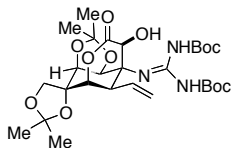
Difference nOe spectrum for lactone 14.



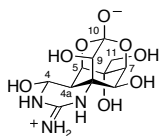
**Oxazolidinone 17:** TLC  $R_f = 0.41$  (4:1  $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  6.06 (br s, 1H), 5.27 (s, 1H), 4.32 (d, 1H,  $J = 10.0$  Hz), 4.23 (t, 1H,  $J = 1.2$  Hz), 4.20 (dd, 1H,  $J = 6.4, 1.2$  Hz), 4.09 (d, 1H,  $J = 10.1$  Hz), 3.81 (d, 1H,  $J = 6.3$  Hz), 3.09 (dt, 1H,  $J = 11.6, 4.6$  Hz), 2.90 (td, 1H,  $J = 11.3, 3.6$  Hz), 2.39 (ddd, 1H,  $J = 10.0, 4.1, 0.8$  Hz), 1.95–1.88 (m, 1H), 1.58–1.51 (m, 1H), 1.27 (s, 3H), 1.15 (s, 3H), 1.04 (s, 3H), 0.94 (s, 3H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  163.9, 156.8, 112.1, 110.3, 79.4, 79.2, 78.7, 75.0, 71.9, 68.0, 61.8, 41.2, 33.4, 29.7, 26.7, 25.8, 25.6, 24.7 ppm; IR (thin film)  $\nu$  3293, 2991, 2925, 1766 (br), 1376, 1254, 1211, 1154, 1060, 989, 913, 857, 833, 795, 734  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{18}\text{H}_{24}\text{NO}_8\text{Cl}$  417.1190 found 402.0962 ( $\text{M}^+ - \text{CH}_3$ ).



**Protected "Tetradamine" 18:** Following ring opening ( $\text{K}_2\text{CO}_3$ ,  $\text{THF}/\text{MeOH}$  23  $^\circ\text{C}$ ) of oxazolidinone **17**, a small coupling constant of  $\sim 1$  Hz was measured between protons  $\text{H}_{4a}$  and  $\text{H}_9$  in the  $^1\text{H}$  NMR spectrum of **18**. The stereochemical integrity of the C9 center is confirmed by the observance of this W-type coupling. W-Coupling between  $\text{H}_{4a}$  and  $\text{H}_9$  is also noted in the  $^1\text{H}$  NMR spectrum of the natural product (see: Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H. *J. Am. Chem. Soc.* **1988**, *110*, 2344-2345).



**Protected TTX Precursor:** TLC  $R_f = 0.34$  (4:1 hexanes/ $\text{EtOAc}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  11.31 (s, 1H), 9.00 (s, 1H), 6.11 (ddd, 1H,  $J = 17.3, 10.4, 7.9$  Hz), 5.70 (d, 1H,  $J = 6.3$  Hz), 5.27 (d, 1H,  $J = 18.4$  Hz), 5.24 (d, 1H,  $J = 10.9$  Hz), 4.67 (s, 1H), 4.46 (s, 1H), 4.39 (d, 1H,  $J = 9.9$  Hz), 4.38–4.36 (m, 2H), 4.24 (d, 1H,  $J = 9.9$  Hz), 3.26 (br s, 1H), 1.51 (s, 3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.44 (s, 6H), 1.36 (s, 3H) ppm;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  169.3, 162.5, 154.3, 152.8, 133.0, 118.8, 111.4, 109.5, 83.2, 81.0, 78.8 (2), 78.4, 74.2, 69.0, 67.3, 59.6, 36.3, 28.2, 28.1, 27.1, 26.1, 25.0, 24.7 ppm; IR (thin film)  $\nu$  3259, 2981, 2933, 1753, 1730, 1643, 1619, 1421, 1370, 1337, 1322, 1282, 1253, 1137, 1099, 1073, 1058, 844  $\text{cm}^{-1}$ . HRMS (EI) calcd for  $\text{C}_{28}\text{H}_{43}\text{N}_3\text{O}_{11}$  597.2898 found 598.2981 ( $\text{MH}^+$ ).



(-)-**Tetrodotoxin**: TLC  $R_f = 0.69$  (2:2:1 *t*-BuOH/H<sub>2</sub>O/AcOH); <sup>1</sup>H NMR (1% CF<sub>3</sub>CO<sub>2</sub>D, 4% CD<sub>3</sub>CO<sub>2</sub>H/D<sub>2</sub>O, 500 MHz) δ 5.50 (d, 1H, 9.7 Hz), 4.30 (d, 1H,  $J = 1.6$  Hz), 4.26 (br s, 1H), 4.09 (br s, 1H), 4.06 (d, 1H,  $J = 12.2$  Hz), 4.02 (d, 1H,  $J = 12.3$  Hz), 3.96 (s, 1H), 2.35 (d, 1H,  $J = 9.4$  Hz) ppm; <sup>13</sup>C NMR (1% CF<sub>3</sub>CO<sub>2</sub>D, 4% CD<sub>3</sub>CO<sub>2</sub>H/D<sub>2</sub>O, 600 MHz) determined by HSQC or HMBC(\*) δ 110.8\*, 79.8, 75.3, 74.0, 73.0, 71.3\*, 71.1 65.7, 59.7\*, 40.9 ppm; HRMS (EI) calcd for C<sub>11</sub>H<sub>17</sub>N<sub>3</sub>O<sub>8</sub> 319.1016 found 320.1093 (MH<sup>+</sup>).

### Comparative NMR Data:

Position	Synthetic TTX		Natural TTX	
	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H
2	*	–	156.6	–
4	75.3	5.50 (d $J = 9.7$ Hz)	75.1	5.50 (d, $J = 9.4$ Hz)
4a	40.9	2.35 (d $J = 9.4$ Hz)	40.7	2.35 (d, $J = 9.5$ Hz)
5	74.0	4.26 (br s)	73.8	4.25 (br s)
6	71.3	–	71.5	–
7	79.8	4.09 (br s)	79.7	4.08 (t $J = 1.8$ Hz)
8	73.0	4.30 (d $J = 1.6$ Hz)	72.8	4.30 (d $J = 1.5$ Hz)
8a	59.7	–	59.7	–
9	71.1	3.96 (s)	70.9	3.96 (s)
10	110.8	–	110.8	–
11	65.7	4.02 (d, $J = 12.3$ Hz) 4.06 (d, $J = 12.2$ Hz)	65.5	4.02 (d, $J = 12.6$ Hz) 4.04 (d, $J = 12.6$ Hz)

NMR data for natural (-)-TTX, see: Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H.  
*J. Am. Chem. Soc.* **1988**, *110*, 2344-2345.

Samples were referenced to CHD<sub>2</sub>CO<sub>2</sub>D = 2.06 ppm, <sup>13</sup>CHD<sub>2</sub>CO<sub>2</sub>D = 22.4 ppm

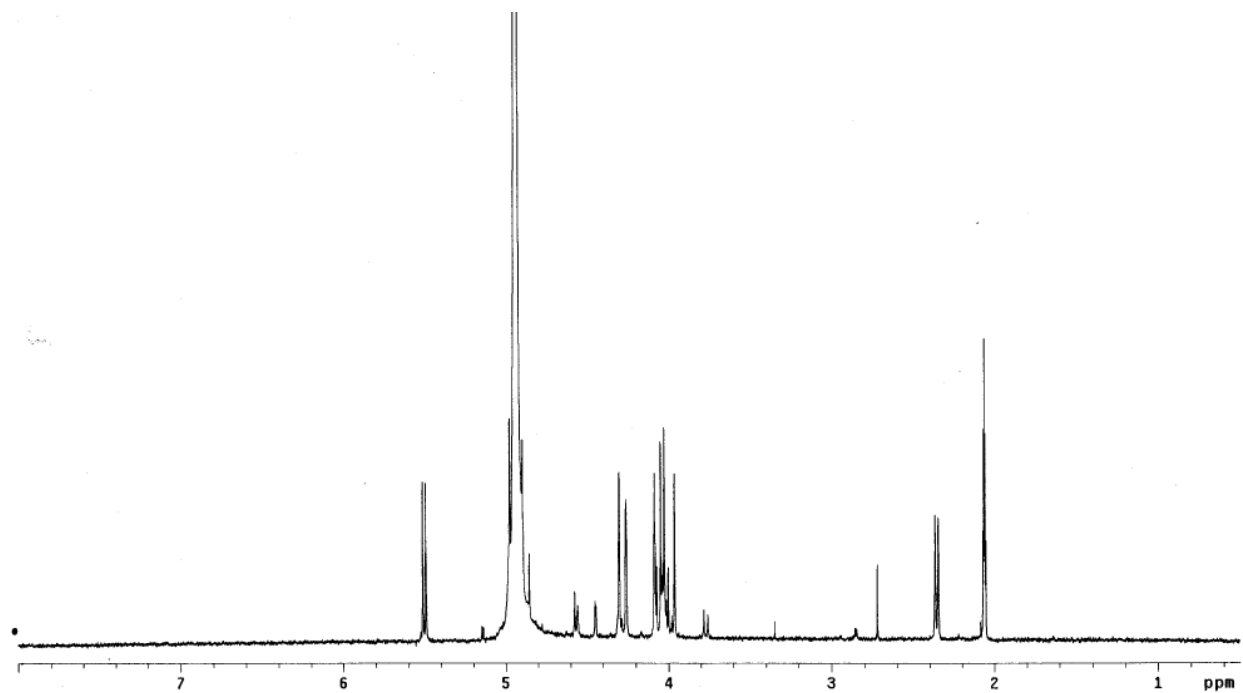
\*carbon signal not observed in HMBC experiment

**<sup>1</sup>H NMR Spectra for Synthetic and Natural (-)-Tetrodotoxin**

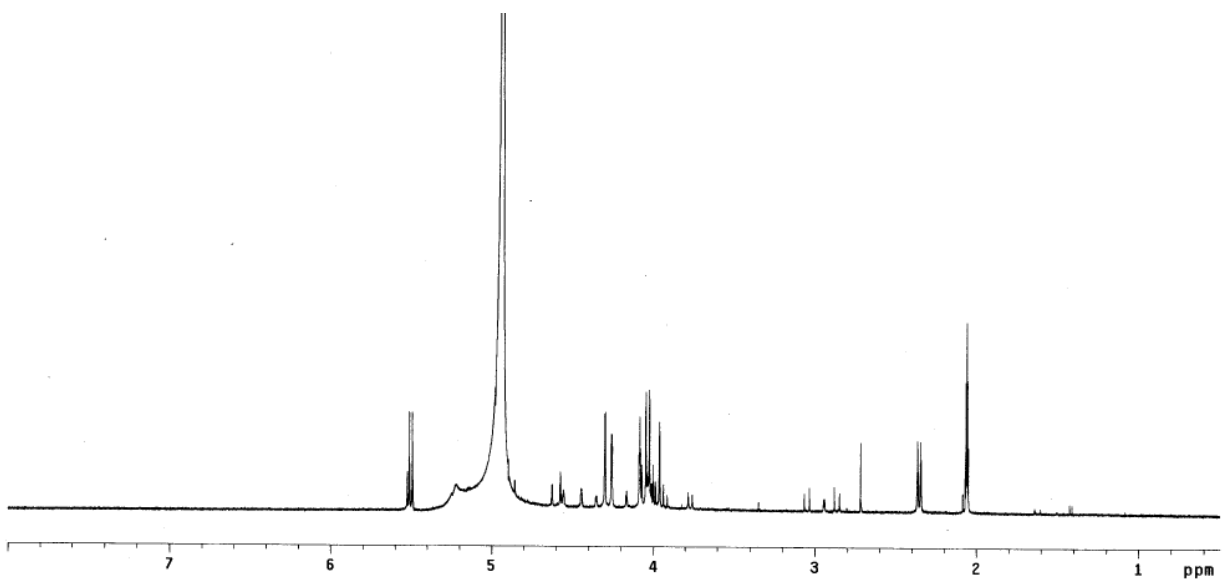
1% CF<sub>3</sub>CO<sub>2</sub>D, 4% CD<sub>3</sub>CO<sub>2</sub>H/D<sub>2</sub>O, 500 MHz

referenced to CHD<sub>2</sub>CO<sub>2</sub>D = 2.06 ppm

**Synthetic (-)-TTX**

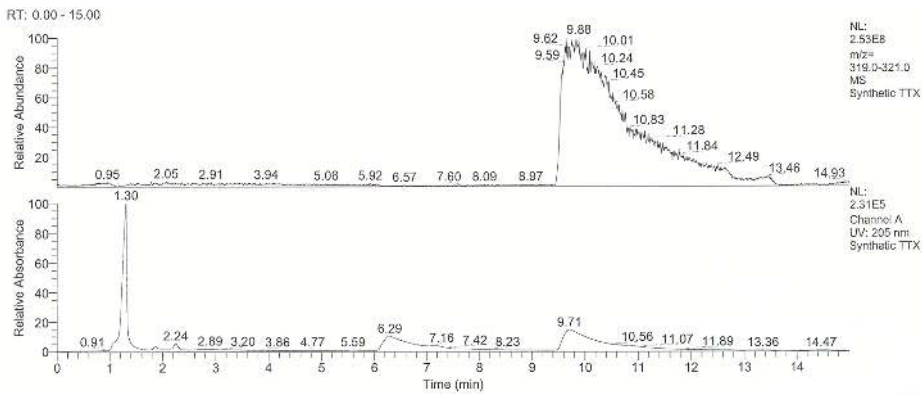


**Natural (-)-TTX**

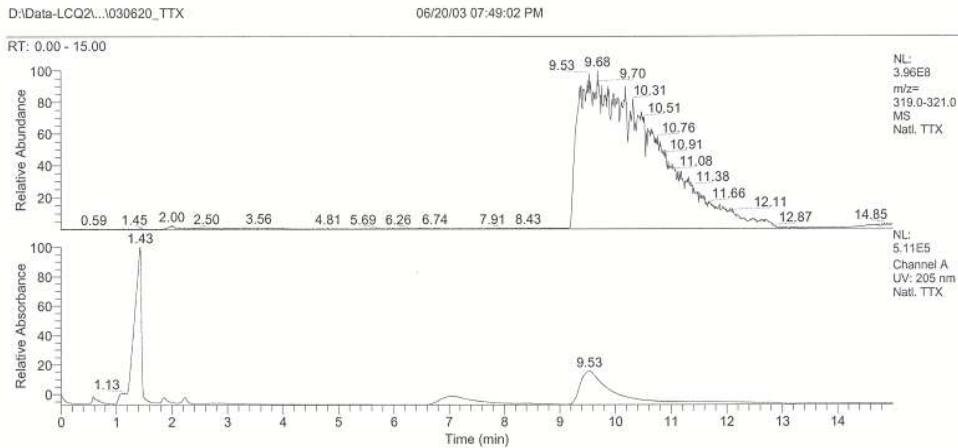


## HPLC-MS Data

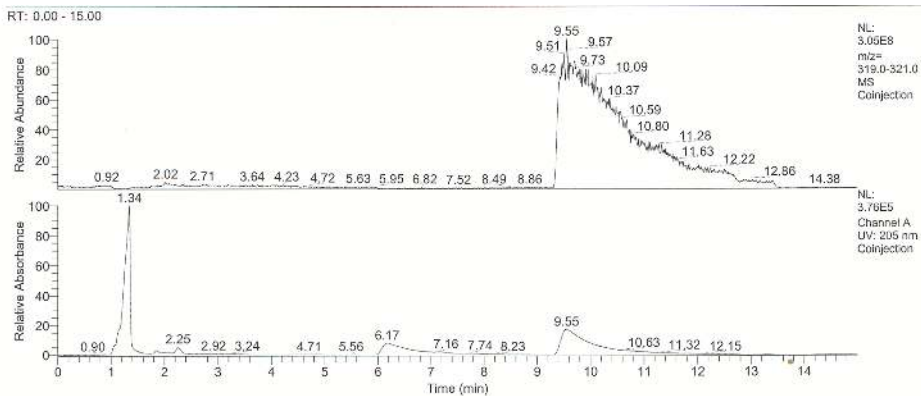
### Synthetic (-)-TTX



### Natural (-)-TTX



### Co-injection (1:1 Mixture)



### Acquisition parameters:

Flow rate: 1 mL/min; 1% MeOH/99% 5 mM HCO<sub>2</sub>NH<sub>4</sub>/HCO<sub>2</sub>H buffer (pH 8.2)

Phenomenex Spherisorb 5-CN column (4.6 x 150 mm), 23 °C

MS: m/z = 319.0–321.0 (upper traces)

UV: 205 nm (lower traces)