SUPPORTING INFORMATION

Development of Certified Reference Materials for Diarrhetic Shellfish Poisoning Toxins. Part 1: Calibration Solutions

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SI-1: Structure Characterization of 19-epi-DTX2 by NMR

1D-^{1}H spectrum showed resonances consistent with DTX2, notably the vinyl protons at lower field than the OH resonance of the solvent and the proper number of methyl resonances (Figure S1). Proton and ^{13}C chemical shifts are shown in Fig. S2A and S2B respectively. The proton resonances were identical to published data for DTX2 (1) except for significant deviations in chemical shift between positions C15 and C23, with a maximum difference at C18 and C23, as shown in Fig. S3 for both the ^{1}H and ^{13}C. This is consistent with the published reports of the 19-epimer of OA (2). ^{13}C resonances were determined from the HSQC (Fig. S4) and HMBC (Fig. S5) spectra. Analysis of the HSQC spectrum yielded 5 methyl resonances, 16 methylene resonances and 16 methine resonances. The HMBC spectrum gave chemical shifts for a further 7 quaternary carbons.

Interpretation of the 2D-^{1}H TOCSY spectrum (Fig. S6) revealed the following spin systems:

1. 4.06, 3.36, 1.93, 1.80, 1.70, 1.67, 1.35
2. 5.89, 5.66, 4.53, 3.88, 2.47, 2.39, 2.07, 2.04, 1.91, 1.77, 1.66, 1.13
3. 4.16, 3.66, 3.04, 2.03, 1.93, 1.83, 1.53
4. 4.08, 3.97, 3.41, 1.82, 1.73, 1.67, 1.60, 1.53, 1.42, 1.37, 1.27, 1.17, 0.96
5. 3.69, 3.51, 2.17, 1.78, 1.61, 1.30, 1.26, 0.99

Isolated resonances were also observed at 1.73, 1.31 ppm. Vinyl resonance at 5.26 ppm also showed TOCSY correlations to the methyl resonance at 1.73 ppm and the resonances at 2.04 and 1.77 ppm. The vinyl resonances at 5.37 and 5.05 ppm showed TOCSY correlations to resonances at 4.16, 3.66 and 3.04 ppm. Analysis of the COSY (Fig. S7), TOCSY (Fig. S6) and HMBC (Fig. S5) spectra revealed the same connectivity as in DTX2. Figure S8 shows the TOCSY spin

systems as bold bonds as well as the key HMBC correlations used to connect the spin systems, which indicates that the new compound is an isomer of DTX2.

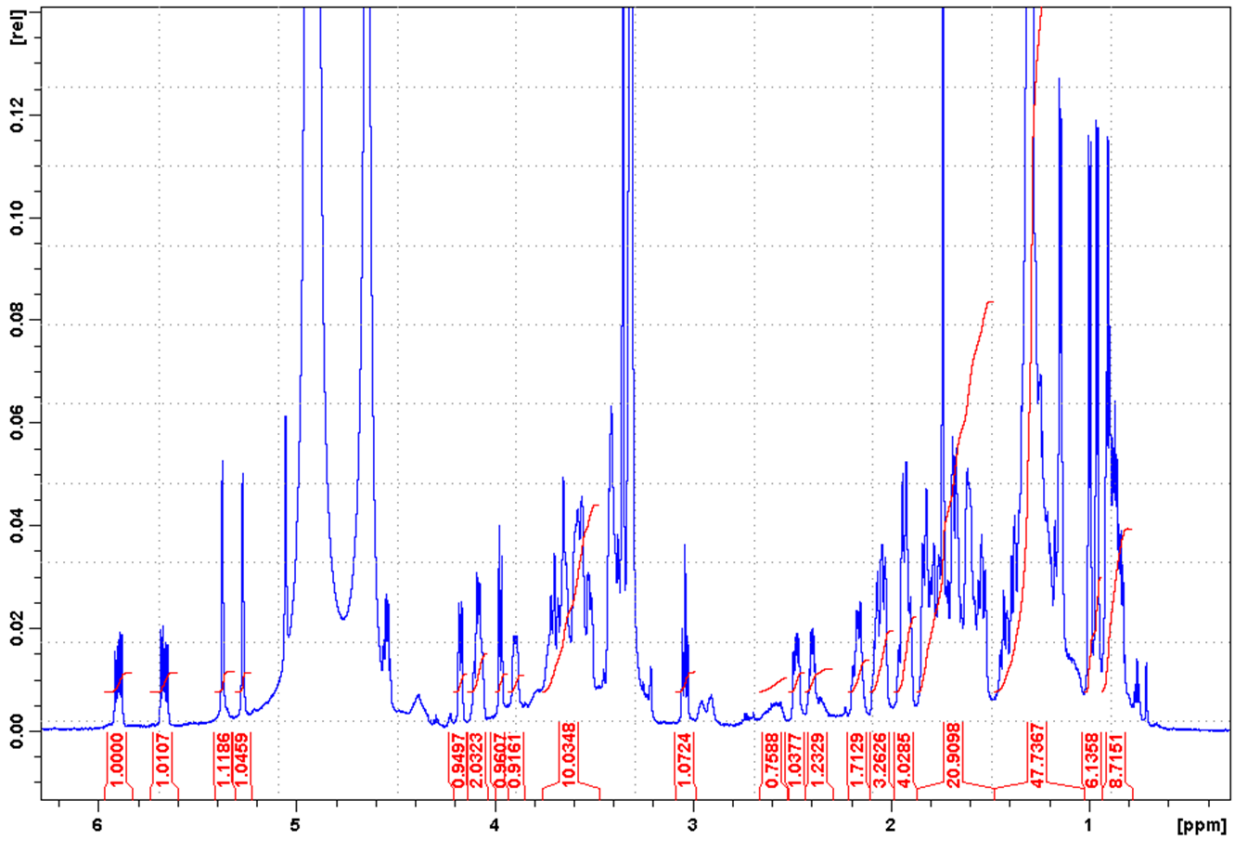


Figure S1. 1D-^{1}H spectrum of 19-*epi*-DTX2 in CD_3OD (^{1}H: 700 MHz).

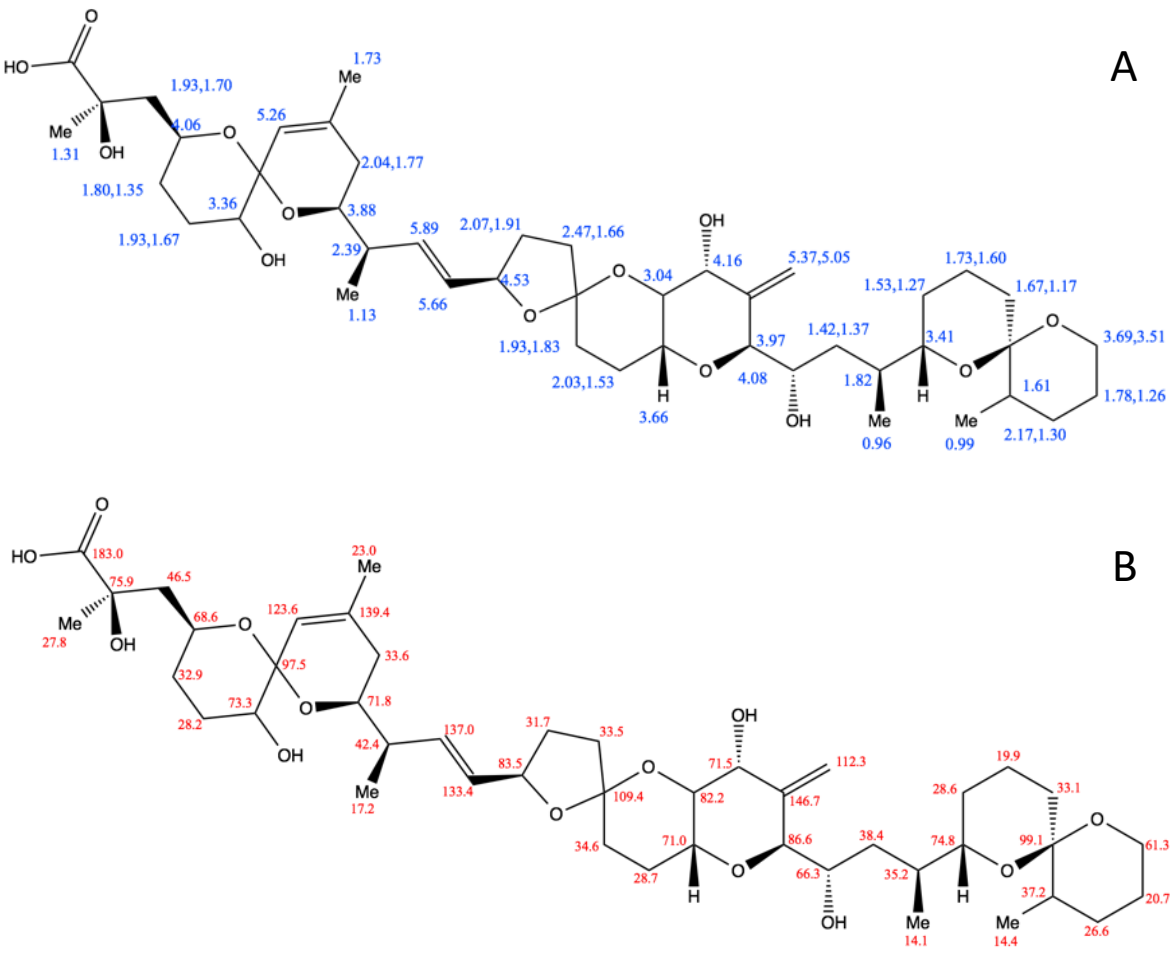


Figure S2. Structure of 19-*epi*-DTX2 showing ^{1}H (A) and ^{13}C (B) chemical shift assignments.

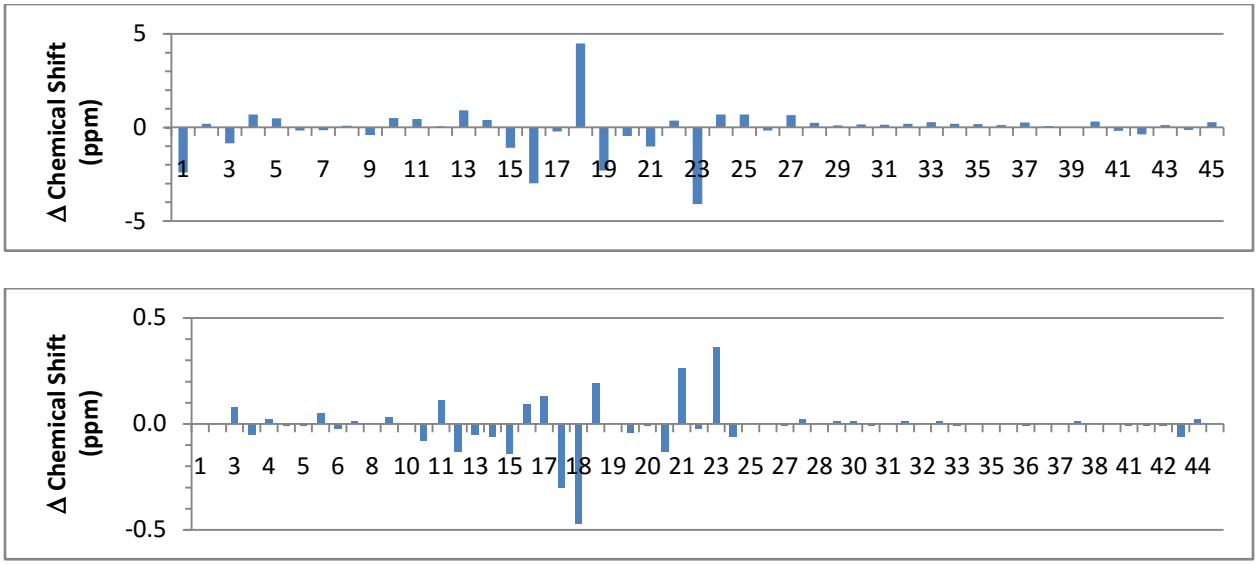


Figure S3. Plot of the difference in ^{13}C chemical shift (top) and ^{1}H chemical shift (bottom) between DTX2 and 19-*epi*-DTX2. Note the largest difference is at the protons and carbons of C18 and C23.

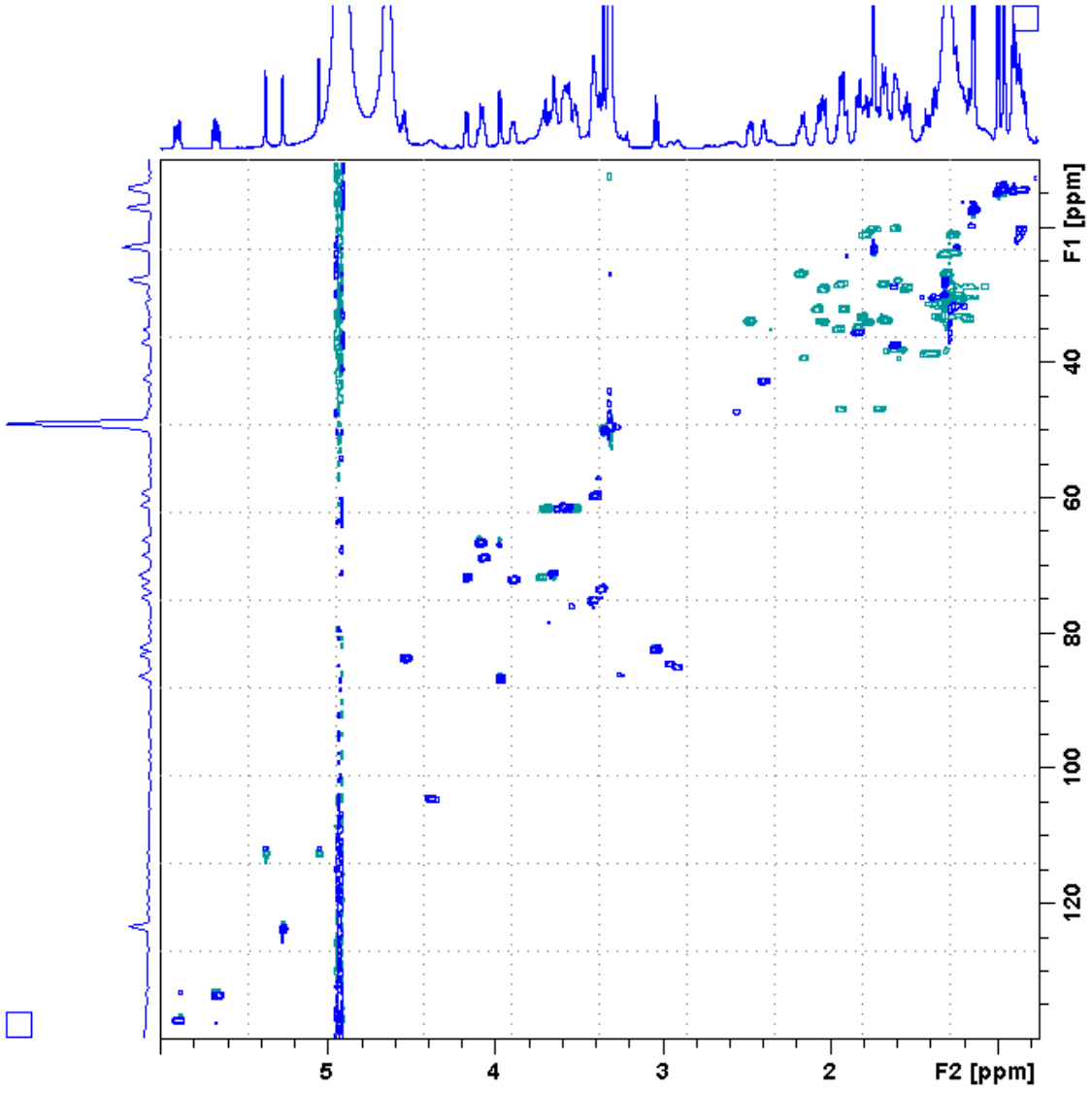


Figure S4. ^{1}H-^{13}C HSQC spectrum of 19-*epi*-DTX2 acquired with multiplicity sorting.

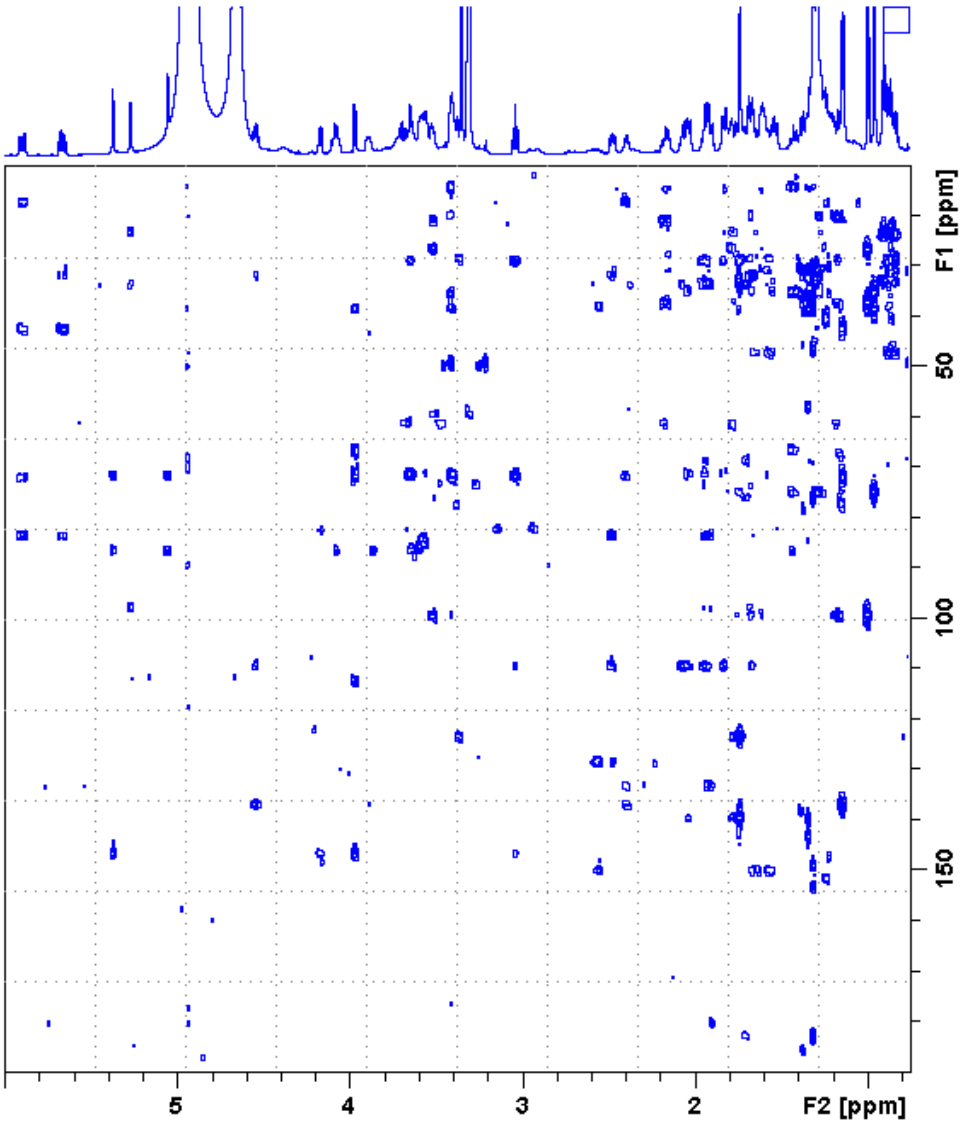


Figure S5. ^{1}H-^{13}C HMBC spectrum of 19-*epi*-DTX2 acquired with 60 ms mixing time time in CD_3OD.

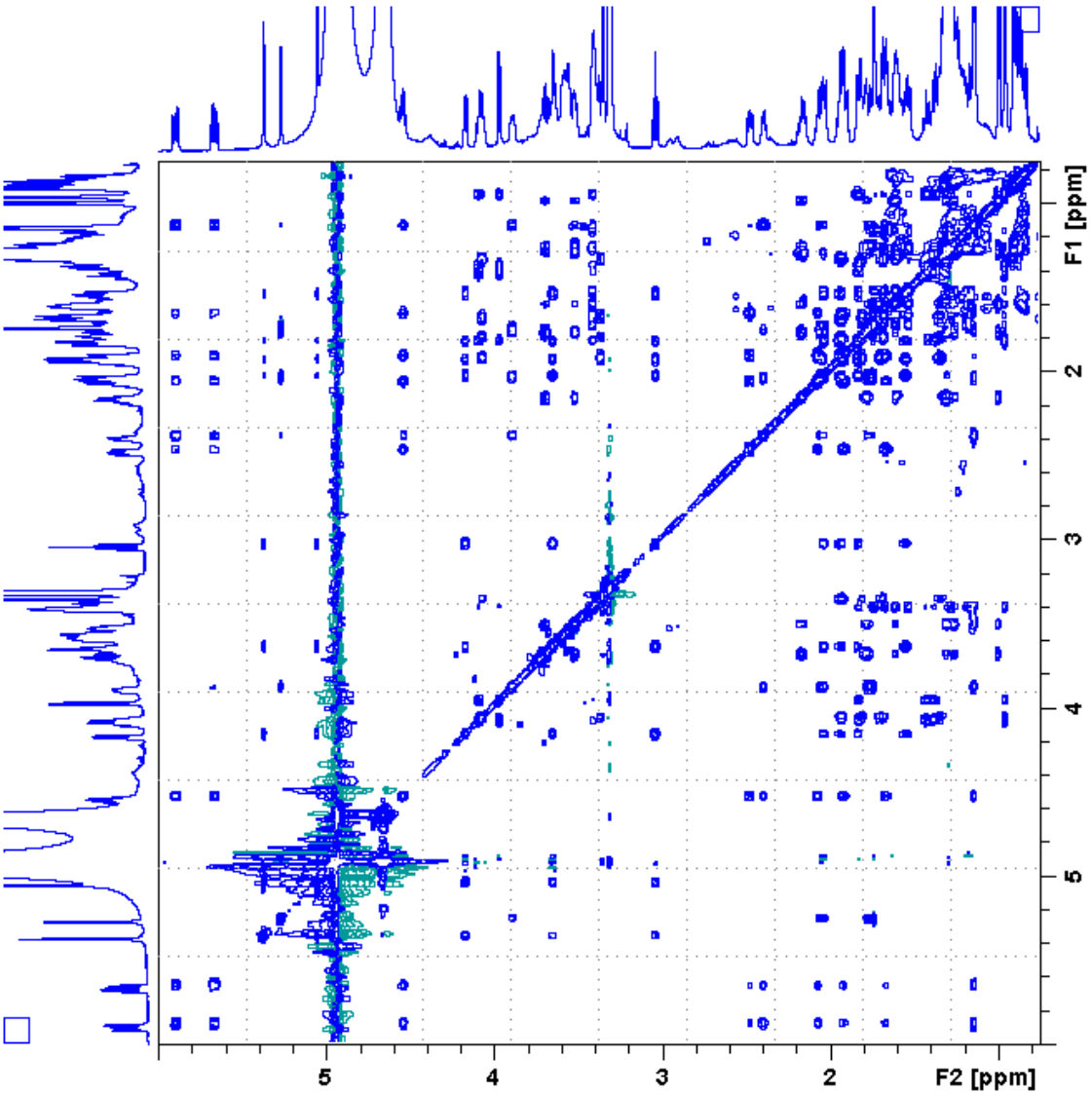


Figure S6. TOCSY spectrum of 19-*epi*-DTX2 with 120 ms mixing time in CD_3OD.

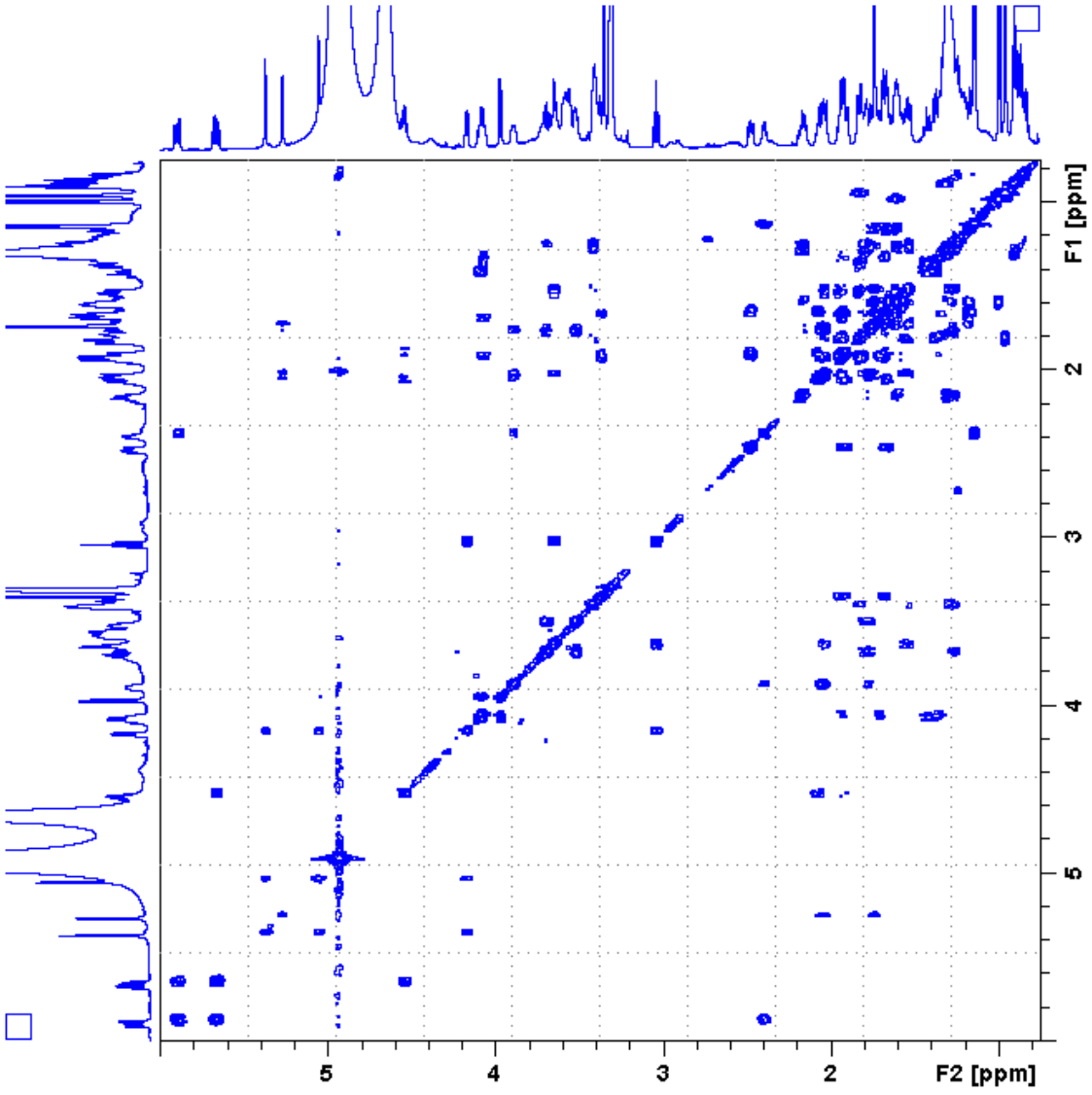


Figure S7. DQF-COSY spectrum of 19-*epi*-DTX2 in CD_3OD.

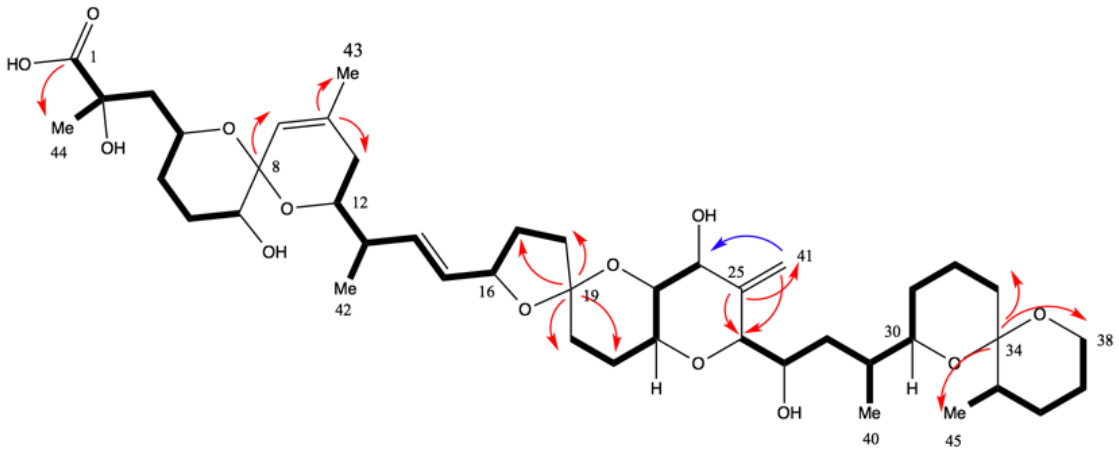


Figure S8. TOCSY spin systems shown as bold bonds. Red arrows indicate the key HMBC correlations (pointing from carbon to proton) used to connect the spin systems. The blue arrow indicates the COSY correlation between the pendant vinyl protons H41 at 5.37 and 5.05 ppm and H24 at 4.16 ppm. The numbering of carbons in the molecule is consistent with Hu *et al*, 1992.

SI-2: High Resolution Mass Spectrometry analysis of DSP toxin CRMs

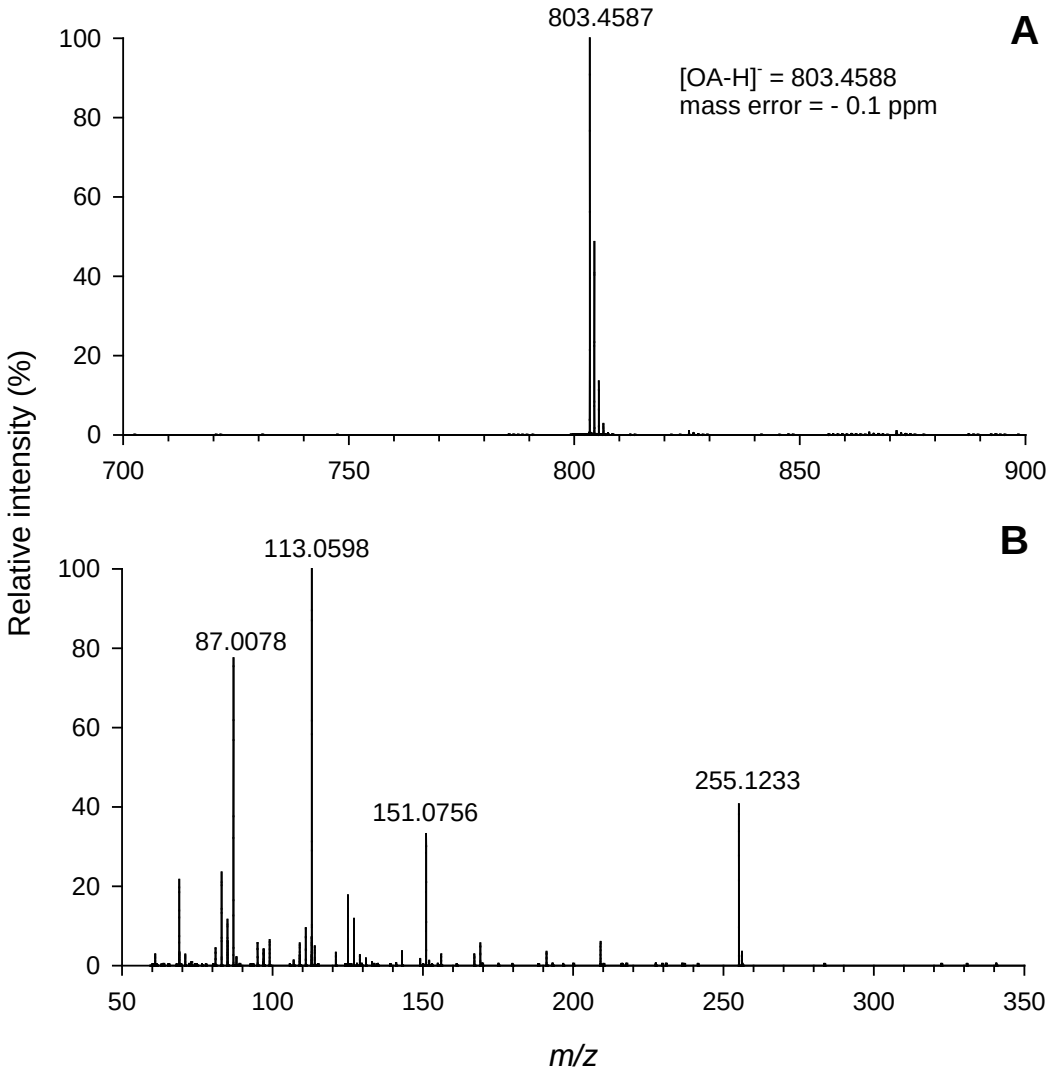


Figure S9. High-resolution full scan (A) and product ion (B) mass spectra of the $[M-H]^-$ ion, *m/z* 803, of OA in CRM-OA-d measured on a Thermo Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization probe. Data acquired in negative ion mode with a -2.7 kV spray voltage, +360 °C capillary temperature, and +250 °C heater temperature. HCD collision energy in (B) was 60 V.

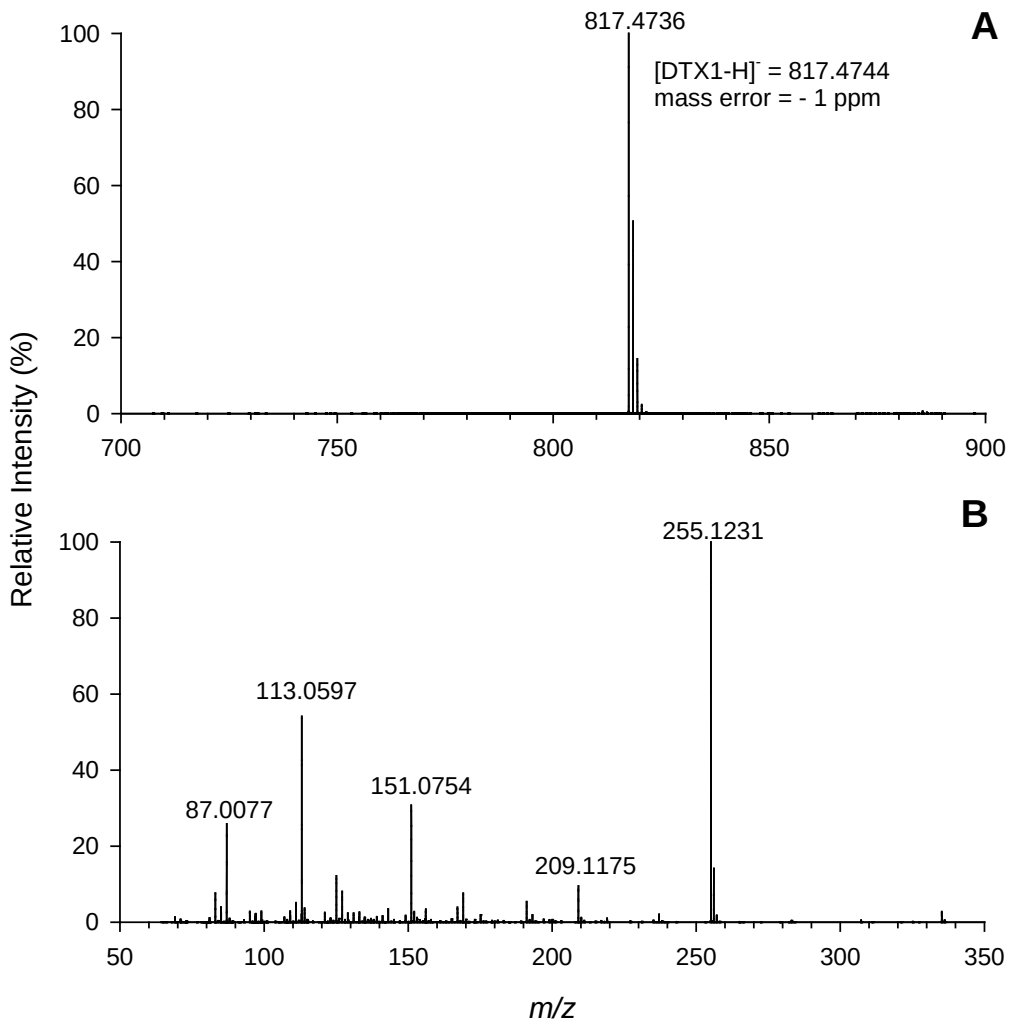


Figure S10. High-resolution full scan (A) and collision induced dissociation (B) mass spectra of the $[M-H]^-$ ion, *m/z* 817, of DTX1 in CRM-DTX1-b measured on a Thermo Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization probe. Data acquired in negative ion mode with a -2.7 kV spray voltage, + 360 °C capillary temperature, and + 250 °C heater temperature. HCD collision energy in (B) was 60 V.

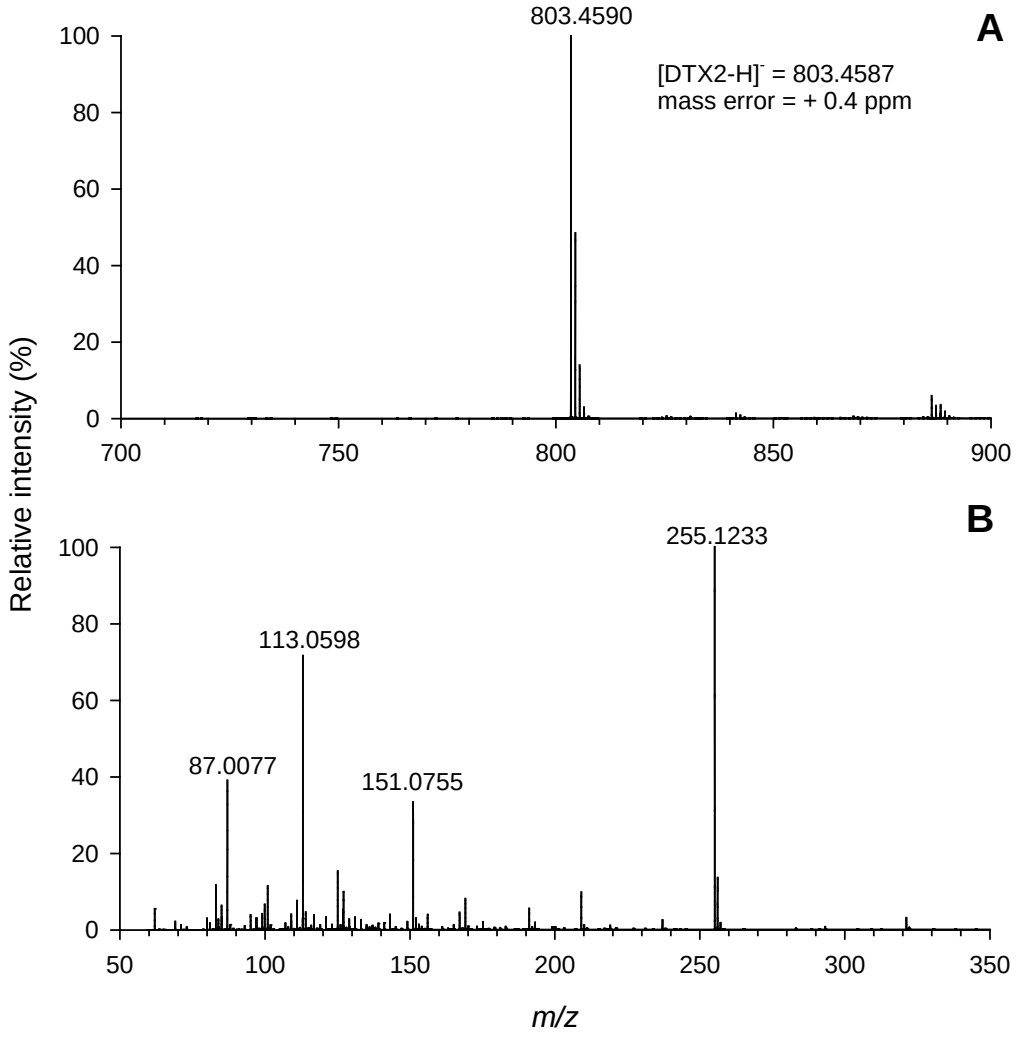


Figure S11. High-resolution full scan (A) and collision induced dissociation (B) mass spectra of the $[M-H]^-$ ion, *m/z* 803, of DTX2 in CRM-DTX2-b measured on a Thermo Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization probe. Data acquired in negative ion mode with a -2.7 kV spray voltage, + 360 °C capillary temperature, and + 250 °C heater temperature. HCD collision energy in (B) was 60 V.

SI-3: Preliminary Stability Studies on OA in Solution

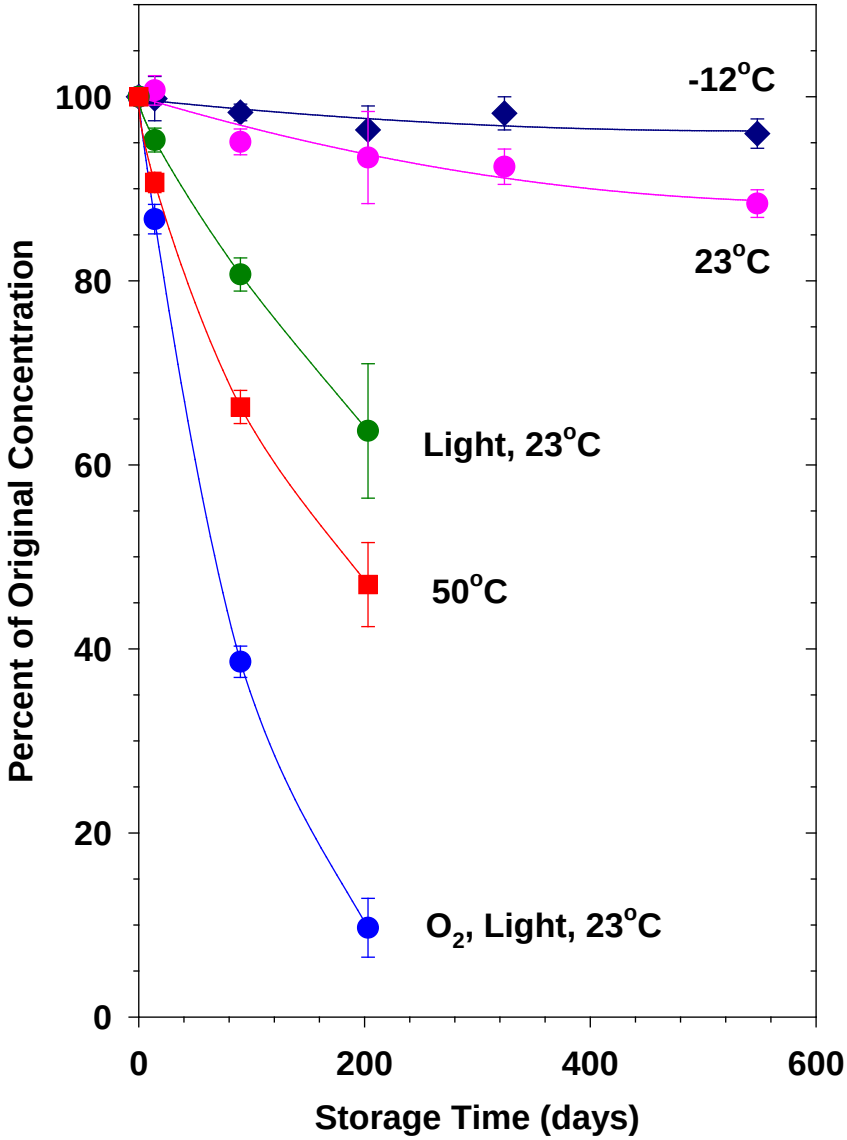


Figure S12. Stability of OA solutions in DMF at a range of temperatures and environmental conditions.

Ion	Elemental Comp.	Calculated m/z	Observed m/z	Δ (ppm)
$[M+Na]^+$	$C_{43}H_{66}O_{11}Na^+$	781.4497	781.4479	-2.3
$[M+H]^+$	$C_{43}H_{67}O_{11}^+$	759.4678	759.4650	-3.7
$[M+H-2H_2O]^+$	$C_{43}H_{63}O_9^+$	723.4467	723.4445	-3.0
Fragment a	$C_{31}H_{48}O_8Na^+$	571.3241	571.3237	-0.7
Fragment b	$C_{13}H_{17}O_3^+$	221.1172	221.1176	1.8

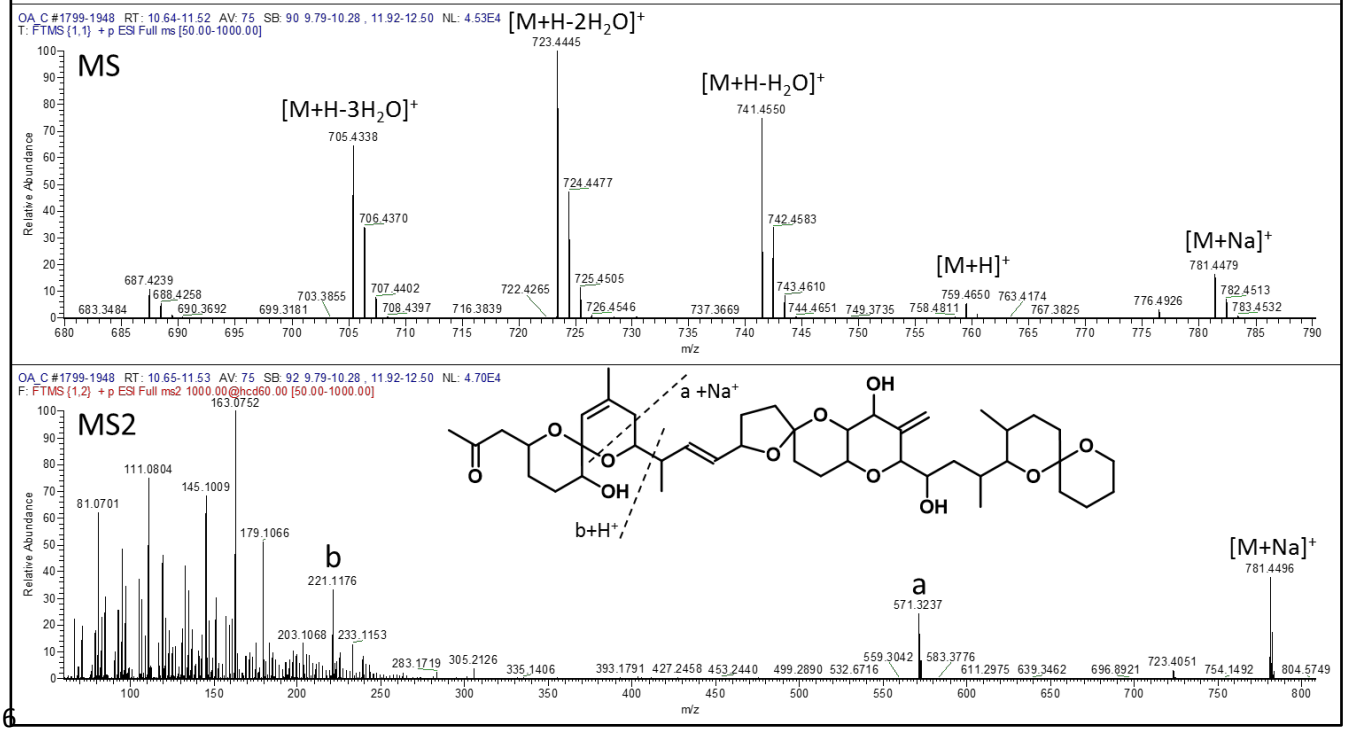
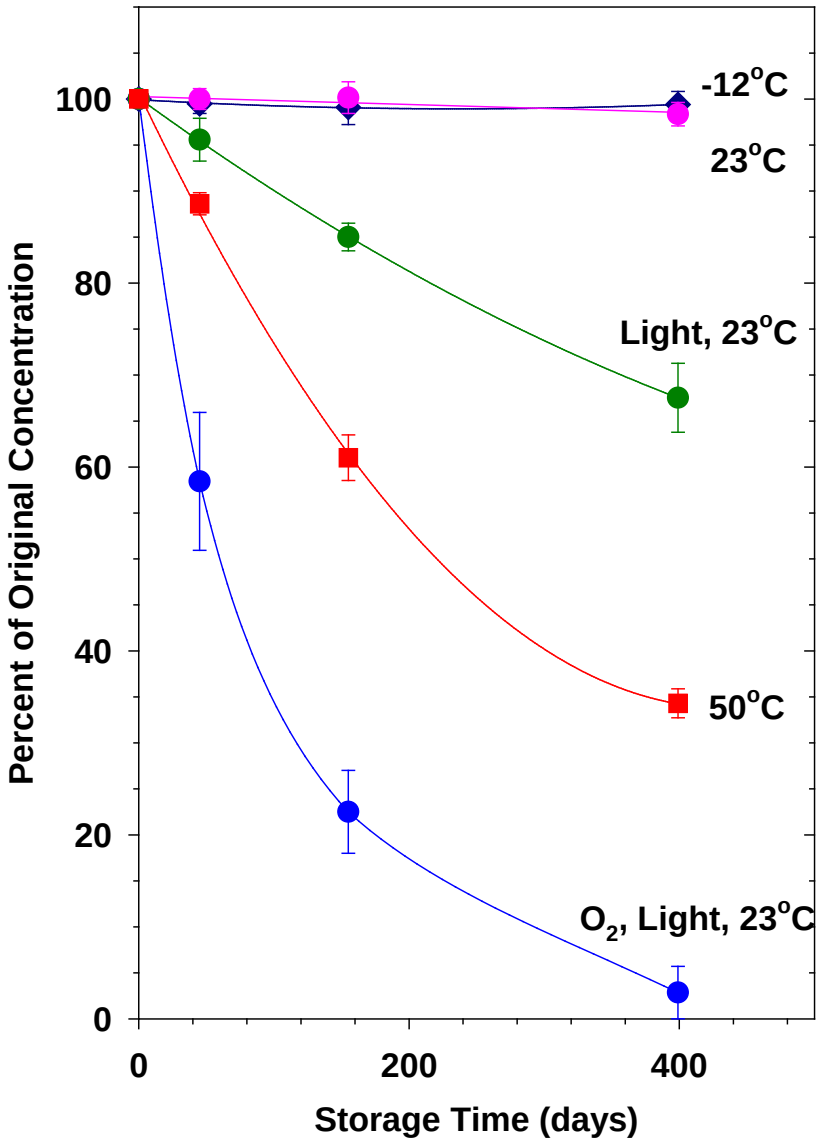


Figure S13. High resolution mass spectra of the OA degradation product observed in a DMF solution exposed to light and oxygen at room temperature for one week. The product was tentatively identified as the ketone analog formed by elimination of formic acid.



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127 **Figure S14**. Stability of OA solutions in MeOH at a range of temperatures and environmental
128 conditions.

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SI-4: Additional Selected Reaction Monitoring Conditions

Table S1: Selected Reaction Monitoring settings used for quantitative LC-MS/MS analysis of DSP toxins.

Analyte	*m/z* Precursor > *m/z* Product	Declustering Potential (V)	Collision Energy (V)
OA	803.5>255.1	-80	-65
	803.5>113.1	-80	-85
DTX1	817.5>255.1	-70	-70
	817.5>113.1	-70	-90
DTX2	803.5>255.1	-80	-65
	803.5>113.1	-80	-85

Parameter	Setting	Parameter	Setting
Dwell (msec)	125	CUR (psi)	20
GS1 (psi)	50	GS2 (psi)	50
IS (V)	-4500	CAD (V)	-3
EP (V)	-10	CXP (V)	-6

SI-5: LC-FLD of ADAM Derivatized DSP toxin CRMs

SI-5.1 ADAM Derivatization

Reactions were carried out under yellow or reduced light. The ADAM precursor, 9-anthraldehyde hydrazone, was prepared from 9-anthralaldehyde and hydrazone hydrate according to the method of Nakaya et al. (3). Solutions of 9-anthraldehyde hydrazone (35 mM), *N*-chlorosuccinimide (35 mM) and quinuclidine (70 mM) were prepared in THF. To prepare the ADAM reagent, equivalent volumes (500 µL) of all three solutions were mixed in an amber glass vial and allowed to react at ambient temperature for 1 hour prior to sample derivatization. A 35 µL aliquot of the sample was placed in a 1.5 mL amber vial with 100 µL of the ADAM reaction reagent. The reaction mixture was placed in a sonication bath (Model 1510, Branson, Danbury, CT, USA) for 10 min at 37 °C and then transferred to a mutlitherm thermal reaction chamber (Model H5000-HC, Benchmark Scientific, Sayreville, NJ, USA) for 2.5 hours at 37 °C. Samples were sonicated for an additional 10 min before drying down using a rotary vacuum drier (Model SPD2010-220, Savant Instrument Inc., Holbrook, NJ, USA) and then reconstituted in 300 µL n-hexanes: chloroform (1:1) for SPE.

SI-5.2 Solid Phase Extraction of Derivatized Samples

Derivatized samples were cleaned up using the SPE procedure published previously (4). Glass SPE tubes (7 mL) equipped with a Teflon frit were packed with 0.5 g of activated silica and placed on a vacuum manifold. The columns were conditioned with 6 mL of chloroform and 3 mL n-hexanes:chloroform (1:1). The columns were not allowed to become dry from this point on. The ADAM derivatized samples were transferred onto the column and vials were rinsed with 2 × 300 µL of n-hexanes: chloroform (1:1) and passed slowly through the column at approx. 1 drop/sec. The columns were then washed with 5 mL of n-hexanes: chloroform (1:1) followed by 5 mL of chloroform (containing 1.15 % ethanol). Samples were eluted with 5 mL of MeOH: chloroform (1.5:8.5). Eluted fractions were evaporated under nitrogen and reconstituted with 2 mL of MeOH.

SI-5.3 Liquid Chromatography with fluorescent detection (LC-FLD)

LC-FLD analysis was carried out on an Agilent 1200 series LC quipped with a fluorescence detector (model no. G1321A) operated with excitation/emission wavelengths of 254/412 nm. A 50 x 2 mm Luna C18 HST (2.5 µm) (Phenomenex, Torrence, CA USA) was eluted isocratically with mobile phase of (A) DIW and (B) 95% acentonitrile each containing 50 mM formic acid and 2 mM ammonium formate at 70% B and 300 µL/min. The column temperature was 20 °C and an injection volume of 5 µL was used.

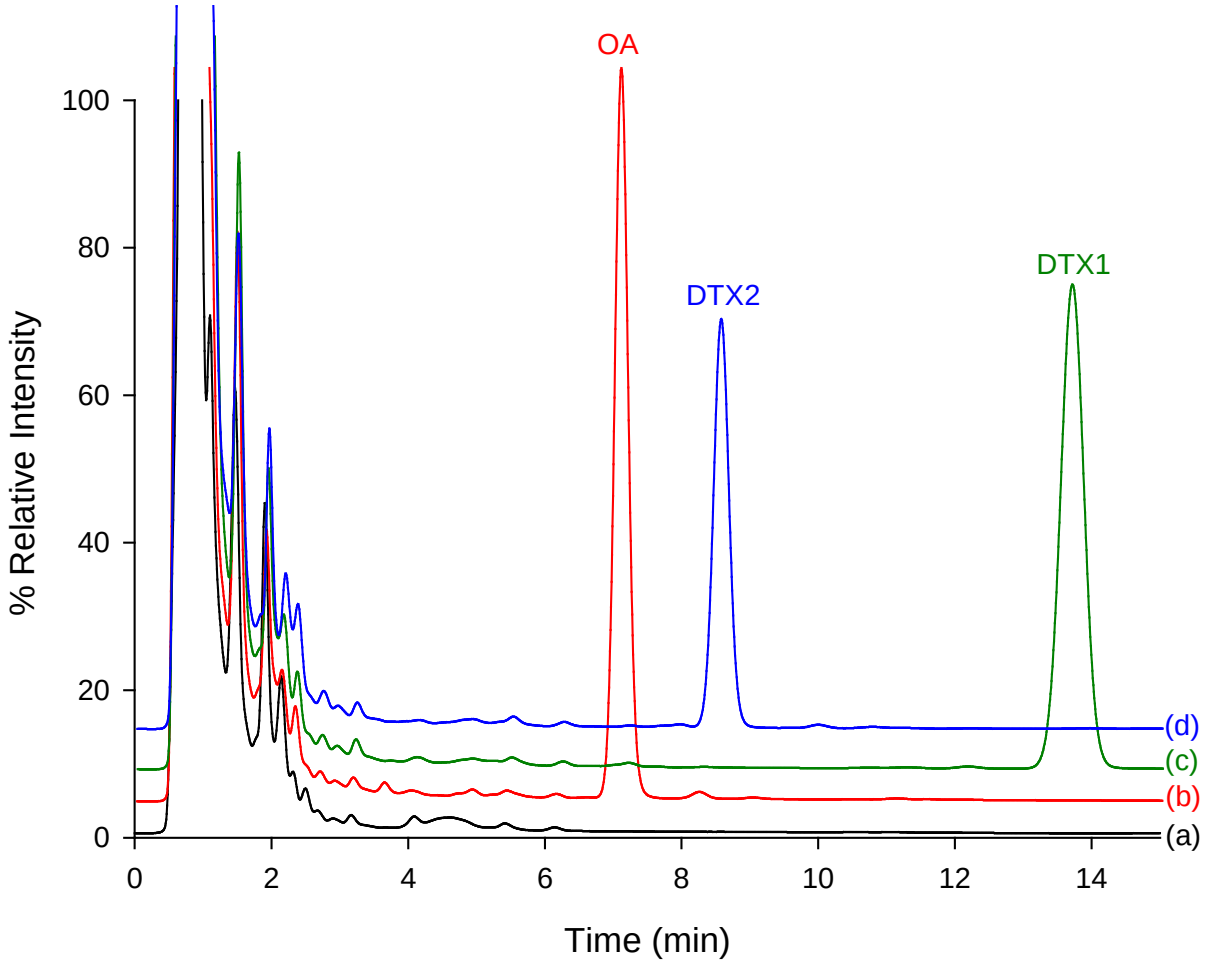

Figure S15: LC-FLD of ADAM derivatized DSP toxin calibration solutions for OA (b), DTX1 (c), DTX2 (d) and a derivatization reagent blank (a).

SI: REFERENCES

(1) Larsen, K., Petersen, D., Wilkins, A.L., Samdal, I.A., Sandvik, M., Rundberget, T., Goldstone, D., Arcus, V., Hovgaard, P., Rise, F., Rehmann, N., Hess, P., & Miles, C.O. (2007) *Chem. Res. Toxicol* .**20**, 868-875

(2) Cruz, P.G., Hernández Daranas, A., Fernandez, J.J., & Norte, M. (2007) *Org. Lett.* **9**, 3045-3048

(3) Nakaya, T., Tomomoto, T., & Imoto, M. (1967) *Bull. Chem. Soc. Japan* **40**, 691-692

(4) Quilliam, M.A., Gago-Martinez, A., & Rodriguez-Vazquez, J.A. (1998) *J. Chromatogr. A* **807**, 229-239