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A Two-Directional Synthesis of (±)-Perhydrohistrionicotoxin

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An entirely two-directional synthesis of (±)-perhydrohistrionicotoxin is presented, utilizing a tandem oxime formation/Michael addition/[3 + 2] cycloaddition as the key step. This approach also constitutes formal syntheses of (±)-histrionicotoxin and (±)-histrionicotoxin 235A.

The histrionicotoxins are a family of 16 spirocyclic alkaloids isolated from the skin extracts of the Columbian "poison arrow" frogs, of the family *Dendrobatidae*.[1] Histrionicotoxin **1** (HTX) and its hydrogenation product, the nonnatural perhydrohistrionicotoxin **2**, are both useful biochemical tools for probing the mechanisms of transsynaptic transmission of neuromuscular impulses.[2] This remarkable biological activity, in combination with the parent compound's low abundance in nature (less than 200 μg is isolated per frog skin) and challenging azaspirocyclic framework, has prompted considerable synthetic interest over the last few decades, culminating in three syntheses of **1** and numerous syntheses of **2**[3,4] (Figure 1).

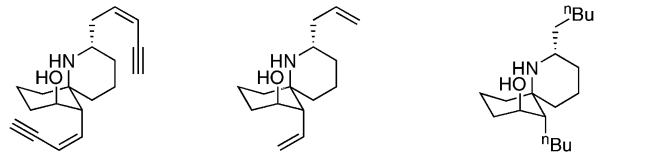
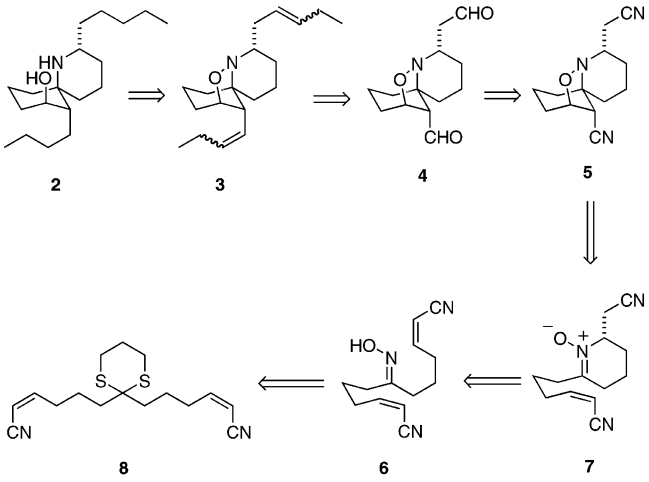


FIGURE 1. Structures of some of the histrionicotoxin family of alkaloids.

Two-directional synthesis[5] and tandem reactions[6] offer the possibility of substantially reducing the number of chemical operations required to synthesize complex target molecules of biological and material interest.[5] We are interested in developing strategies toward natural products that combine these two modern synthetic approaches,[7] and herein we report the entirely two-directional total synthesis of perhydrohistrionicotoxin and the formal syntheses of (±)-histrionicotoxin and (±)-histrionicotoxin 235A.[8]

Our retrosynthesis of perhydrohistrionicotoxin is shown in Scheme 1. It was envisaged that a triple hydrogenation could be used to form **2** from the isoxazoline diene **3**, and this in turn should be available from the dialdehyde **4**. The dialdehyde should be available from a range of different carbonyl derivatives, and we chose the nitrile functionality based on Holmes' seminal work on the regioselectivity of nitrone cycloadditions.[4b] Our key disconnection is from dinitrile **5** to the symmetrical oxime **6**, utilizing a tandem Michael addition/1,3-proton shift/[3 + 2]-cycloaddition[9,10] *via* nitrone **7**. Oxime **6** can be synthesized by functional group interconversion from the disubstituted dithiane **8**, which we can synthesize through a two-directional alkylation approach from dithiane itself.

SCHEME 1. Retrosynthesis of (±)-Perhydrohistrionicotoxin



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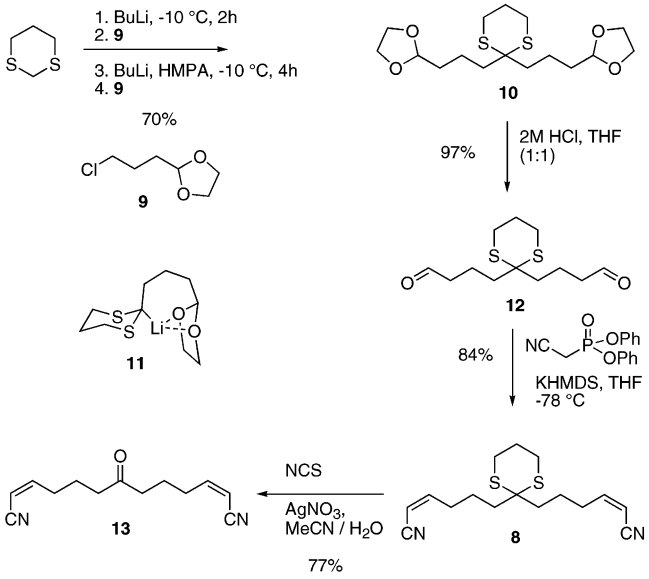
SCHEME 2



Results and Discussion

Our first objective was to doubly alkylate 1,3-dithiane with two suitable four-carbon pieces, which would have suitable functionality to be converted to dialdehyde **12** (Scheme 2). The commercially available 1-(3-chloropropyl)-dioxolane[11] **9** was chosen as this four-carbon unit. Deprotonation of 1,3-dithiane in THF with butyllithium at −10 °C for 2 h, followed by addition of **9** resulted in clean alkylation after 24 h of stirring from −40 °C to room temperature. A second equivalent of butyllithium was added and the reaction mixture stirred at −10 °C for 3 h to effect deprotonation. Addition of a further 1.2 equiv of **9**, followed by stirring for a further 24 h from −40 °C to room temperature, resulted in a complex mixture of products, which included some monoalkylated dithiane (which is green by vanillin-based TLC development), excess **9** (red), and dialkylated dithiane **10** (dark brown). The dialkylated product was isolated in a moderate 54% yield after purification. We surmised that the second alkylation was being hindered by the formation of some sort of aggregate or chelate, possibly of the structure **11**, which was reducing the reactivity of the lithiated monoalkylated dithiane. This was backed up by the lack of acceleration, or enhancement in yield, in the second alkylation seen by changing the halogen leaving group of **9** to bromide or iodide. Thus, a number of deaggregation strategies were investigated, including the use of Lipshutz sodium *tert*-butoxide protocol[12] and addition of DMPU, TMEDA, and HMPA to the reaction mixture. The most successful of these methods was the addition of 2 equiv of HMPA after the second deprotonation and before the second addition of **9**. The reaction mixture was seen to turn dark red some 10−15 min after the addition of HMPA, which also suggests that complexes are being broken up or at least changed in nature. The yield of the reaction in the presence of HMPA was 70%, an average of 83.7% for each alkylation, and thus this method was adopted. The two dioxolane groups of **10** were hydrolyzed selectively over the dithiane group by 2 M aqueous hydrochloric acid in THF to give dialdehyde **12** in near quantitative yield. We evaluated two procedures for the conversion of dialdehyde **12** into the *Z*,*Z*′-di-α,*β*-unsaturated nitrile **8**. Yamamoto's modification of the Peterson reaction of trimethylsilylacetonitrile[13] gave good yields (up to 73%) and up to 12:1 *Z*,*Z*′:*Z*,*E*′ ratios of the alkene geometry but was found to be unpredictable, with some reactions being high yielding and others less so. Zhang's nitrile modification[14] of Ando's protocol[15] for the formation of *Z*-α,*β*-unsaturated esters gave much more reliable yields and equally good stereoselectivity (12:1 *Z*,*Z*′:*Z*,*E*′) and was thus the method of choice. Conversion of the dithiane **8** to the ketone **13** was achieved in 77% yield by treatment with *N*-chlorosuccinimide and silver nitrate in aqueous acetonitrile.[16]

With ketone **13** in hand, we were now set to carry out the oxime formation. Stirring **13** with 1.2 equiv of hydroxylamine hydrochloride and 2 equiv of sodium acetate in methanol for 24 h gave a single new product

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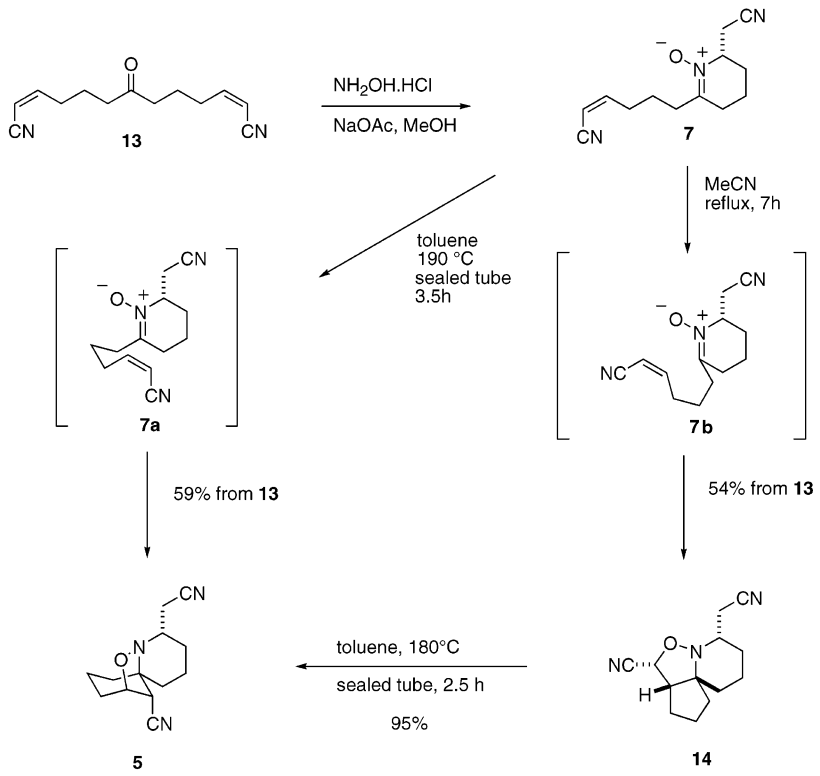
SCHEME 3



by TLC. Addition of saturated sodium hydrogen carbonate and extraction with ethyl acetate yielded crude nitrone **7**, which had formed presumably by oxime formation and subsequent intramolecular Michael addition to one of the α,*β*-unsaturated nitriles, followed by a 1,4-proton shift. This result, although unexpected at the time, is analogous to the findings of Grigg[9] on the formation of nitrones from oxime formation/Michael addition of *ω*-keto-α,*β*-unsaturated esters. Heating the crude nitrone **7** in refluxing acetonitrile induced intramolecular [3 + 2] cycloaddition to give the kinetic [5,5,6]-tricyclic system **14** through the less hindered transition state represented as **7b** (Scheme 3). We also found that the formation of **14** could be achieved in a single pot by the addition of acetonitrile to the hydroxylamine/sodium acetate/methanol solution and heating at reflux for 7 h. We were able to confirm the structure of **14** by X-ray crystal structure analysis (Figure 2). It was found that heating **14** in toluene in a sealed tube at 180 °C for 3 h gave the desired, thermodynamically favored regioisomeric [6,5,6]-tricycle **5** through a presumed retro-[3 + 2] cycloaddition and further cycloaddition through the transition state represented by **7a**. The structure of **5** was also confirmed by X-ray analysis, as shown in Figure 2. This finding is in keeping with the result of Holmes,[4b] who also needed forcing conditions to get the required regioisomer in a similar nitrone cycloaddition reaction. The dinitrile **5** can also be formed directly from the nitrone **7** by heating under the same sealed tube conditions used to convert **14** to **5**. Dinitrile **5** is an advanced intermediate in Holmes' synthesis of histrionicotoxin and histrionicotoxin 235A.[4b,c]

Having set up the histrionicotoxin framework in the cycloaddition, all that was left to accomplish was the conversion of the two nitrile functionalities into butyl

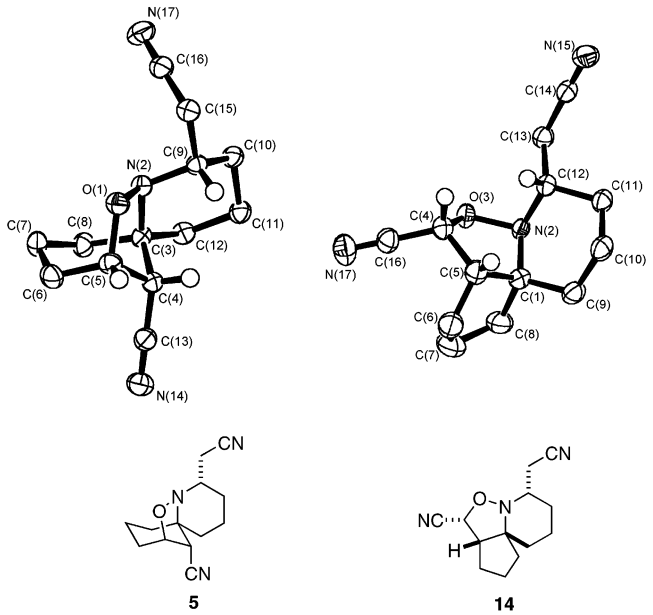
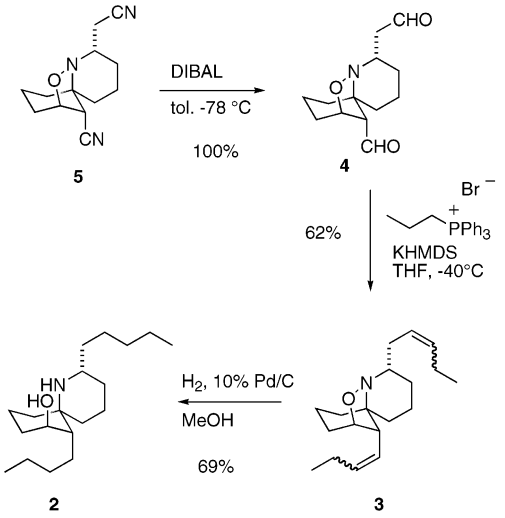


FIGURE 2. X-ray structures of compounds **5** and **14**, indicating the atom numbering schemes. Hydrogen atoms (except at the chiral centers) have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level.

groups and cleavage of the N−O bond. Thus, the two nitriles of **5** were converted into aldehydes by DIBAL reduction/hydrolysis of the imines thus formed. A double Wittig olefination, with the ylide formed by deprotonation of propyltriphenylphosphonium bromide with KHMDS in toluene/THF, gave dialkene **3** in 62% yield as an 8:1 mixture of alkene stereoisomers. Hydrogenation of **3** with 10% palladium on carbon in methanol at atmospheric pressure gave perhydrohistrionicotoxin **2** in nine steps

SCHEME 4. Synthesis of Perhydrohistrionicotoxin 2



and 11.1% overall yield from 1,3-dithiane.In conclusion, we have demonstrated a truly two-directional synthesis of perhydrohistrionicotoxin, in which no desymmetrization was necessary. The key tandem oxime formation/Michael reaction/[3 + 2] cycloaddition sequence generated the spirocyclic framework of the histrionicotoxins from a simple symmetrical precursor, thus demonstrating the power of this strategy for complex molecule synthesis. The investigation of other two-directional synthesis/tandem reaction strategies are ongoing in these laboratories.

Experimental Section

2,2′-Di[3-(2-[1,3]dioxolanyl)propyl]-1,3-dithiane 10. A solution of dithiane (1.10 g, 9.15 mmol) in THF (15 mL) was cooled to −40 °C under argon and treated with butyllithium (6.3 mL of a 1.6 M solution in hexanes). The solution was stirred from −40 to −20 °C over 2 h and then recooled to −78 °C and treated with 2-(3-chloropropyl-[1,3]-dioxolane) (1.2 mL, 1.37 g, 9.10 mmol). The reaction was allowed to warm to room temperature over 16 h and then recooled to −30 °C and treated with butyllithium (7 mL of a 1.6 M solution in hexanes). The solution was stirred for 10 min at −30 °C and then treated with dry HMPA (3.2 mL, 3.26 g, 18.19 mmol). It was noted that the solution turned a deep red color. The solution was stirred at −30 °C for 2 h, and then 2-(3-chloropropyl-[1,3]-dioxolane) (1.4 mL, 1.60 g, 10.62 mmol) was added and the solution allowed to warm to room temperature over 18 h. Water (30 mL) and diethyl ether (40 mL) were then added, and the organic layer was washed with brine (30 mL), dried over magnesium sulfate, and evaporated. Purification by column chromatography over silica gel eluting with 5:1 hexane/ethyl acetate gave compound **10** as a colorless oil (2.240 g, 70%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.87 (2H, t, J = 4.2 Hz), 3.96 (4H, m), 3.82 (4H, m), 2.80 (4H, t, J = 5.7 Hz), 1.94 (2H, m), 1.92 (4H, td, J = 7.8, 1 Hz), 1.51−1.71 (8H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 104.5, 65.0, 53.3, 38.1, 33.9, 29.7, 26.1, 18.8; IR (thin film, cm^{-1}) 1420, 1142; m/z (EI) 348 (M^+, 100%). Anal. Calcd for $C_{16}H_{28}O_4S_2$: C, 55.14; H, 8.10; S, 18.40. Found: C, 54.89; H, 8.05; S, 18.68.

6-[2-(5-Cyano-pent-4-enyl)-[1,3]dithian-2-yl]-hex-2-enenitrile 8. A solution of compound **10** (6.67 g, 19.17 mmol) in THF (300 mL) and 2 M HCl (300 mL) was allowed to stand under an atmosphere of argon for 24 h at room temperature. Diethyl ether (400 mL) was added and the organic layer separated and washed with saturated sodium hydrogen carbonate solution (100 mL) and brine (100 mL). The organics were dried over magnesium sulfate and evaporated to give the dialdehyde **12** as a colorless oil (4.825 g, 97%), which was used immediately in the next reaction. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.80 (2H, d, J = 1.5 Hz), 2.82 (4H, m), 2.5 (4H, td, J = 6.6 and 1.5 Hz), 1.7−2 (10H, m). A solution of diphenylphosphonoacetonitrile[15] (2.2 g, 8.05 mmol) in THF (20 mL) under an atmosphere of argon was cooled to −78 °C. Potassium hexamethyldisilazide (17 mL of a 0.5 M solution in toluene) was added dropwise over 30 min, and the solution was then allowed to warm to −20 °C over 45 min. The solution was recooled to −78 °C, and a solution of crude dialdehyde **12** (1 g, 3.85 mmol) in THF (10 mL) was added dropwise over 10 min. The reaction was allowed to warm from −78 °C to 0 °C over 6 h. Saturated aqueous ammonium chloride solution (40 mL) and ethyl acetate (60 mL) were then added, and the organic layer was separated, washed with brine, dried over magnesium sulfate, and evaporated. Purification by column chromatography over silica gel, eluting with 4:1 hexanes/ethyl acetate, gave the dinitrile **8** as a colorless oil (1.02 g, 84%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.50 (2H, dt, J = 11.1, 7.5 Hz), 5.38 (2H, td, J = 11.1, 0.9 Hz), 2.81 (4H, t, J = 5.7 Hz), 2.46 (4H, dtd, J = 7.5, 7.2, 1.2 Hz), 1.91 (6H, m), 1.62 (4H, m); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 156.6, 118.2, 102.7, 54.7, 40.0, 33.9, 31.8, 28.1, 25.2; IR (thin film, cm^{-1}) 2218, 1619; m/z (CI) 324 (M + NH_4, 100%), 220 (43%). HRMS (CI) Calcd for $C_{16}H_{26}S_2N_3$ (M + NH_4): 324.1568. Found: 324.1562.

***(Z,Z)*-Tridecan-2,11-diene-7-onedinitrile 13.** To a mixture of *N*-chlorosuccinimide (73 mg, 0.55 mmol) and silver nitrate (105 mg, 0.62 mmol) in acetonitrile (3 mL) and water (0.7 mL) was added a solution of dithiane **12** (40.5 mg, 0.13 mmol) in acetonitrile (0.7 mL). The reaction mixture was stirred at room temperature for 20 min, after which time was added in succession a saturated aqueous solution of sodium dithionite (0.7 mL), a saturated aqueous solution of sodium hydrogen carbonate (0.7 mL), and brine (0.7 mL). The aqueous layer was separated and extracted with dichloromethane (3 × 5 mL). The combined organics were dried over magnesium sulfate, evaporated, and purified by column chromatography over silica gel, eluting with 2:1 hexanes/ethyl acetate to give the ketone **13** as a colorless oil (22 mg, 77%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.47 (2H, dt, J = 10.8, 7.8 Hz), 5.36 (2H, dt, J = 10.8, 1.2 Hz), 2.46 (4H, t, J = 7.5 Hz), 2.42 (4H, dtd, J = 7.8, 7.5, 1.2 Hz), 1.78 (4H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.2, 154.3, 118.0, 100.4, 41.7, 31.3, 22.0; IR (thin film, cm^{-1}) 2219, 1708, 1620; m/z (CI) 234 (M + NH_4, 100%). HRMS (CI) Calcd for $C_{13}H_{20}N_3O$ (M + NH_4): 234.1606. Found: 234.1602.

Dintrile 14. To a solution of ketone **13** (127 mg, 0.582 mmol) in methanol (30 mL) and acetonitrile (15 mL) was added hydroxylamine hydrochloride (60 mg, 0.87 mmol) and sodium acetate (120 mg, 1.46 mmol), and the solution was refluxed for 7 h. The solvent was evaporated and the residue redissolved in dichloromethane (10 mL), which was then washed with brine (10 mL), dried over anhydrous sodium sulfate, and concentrated to give dinitrile **14** as colorless plates after recrystallization from hexane (72 mg, 54%): mp = 80.1−81.7 °C (hexane); ^{1}H NMR (400 MHz; $CDCl_3$) δ 4.9 (1H, d, J = 9.2 Hz), 2.75 (1H, t, J = 7.6 Hz), 2.70 (1H, ddd, J = 16.8, 3.6, 1.2 Hz), 2.6 (1H, m), 2.5 (1H, ddd, J = 8.8, 7.6, 1.2 Hz), 2.1−1.1 (12H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 117.8, 116.0, 78.5, 68.9, 58.3, 50.9, 40.4, 33.0, 29.0, 28.9, 23.6, 23.1, 20.2; IR (thin film, cm^{-1}) 2248, 2218; m/z (ES) 249 [(M + NH_4)$^+$, 100%], 232 (85). HRMS (CI) Calcd for $C_{13}H_{18}ON_3$ (M + H^+) $C_{13}H_{18}ON_3$ 232.1450. Found: 232.1451. **Crystal Data:** $C_{13}H_{17}N_3O$, M = 231.3. monoclinic, space group $C2/c$ (no. 15), a = 28.183(6), b = 9.362(2), c = 18.789(4) Å, β = 98.44(3) °, V = 4903.7(4) Å^3; Z = 16, D_c = 1.253 g/cm^3, F(000) = 1984, T = 140(1) K, μ(Mo Kα) = 0.8 cm^{-1}, λ(Mo Kα) = 0.71069 Å; 10 553 reflections were measured, of which 4305 were unique (R_{int} = 0.116); 2822 were "observed" with $I > 2\sigma I$. Final R-factors: wR_2 = 0.157 and R_1 = 0.093 (1b) for all 4305 reflections weighted $w = [\sigma^2(F_o^2) +$

$(0.0852P)^2]^{-1}$ with $P = (F_o^2 + 2F_c^2)/3$; for the "observed" data only, $R_1 = 0.061$.

Dinitrile 5. A solution of ketone **13** (120 mg, 0.58 mmol) in methanol (5 mL) was treated with hydroxylamine hydrochloride (42 mg, 0.60 mmol) and sodium acetate (100 mg, 1.22 mmol) and stirred at room temperature for 24 h. Saturated aqueous sodium hydrogen carbonate solution (10 mL) was added and the solution extracted with ethyl acetate (3 × 10 mL). The combined organics were dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in toluene (10 mL) and the resulting light yellow solution heated in a sealed tube at 190 °C (oil bath temperature) for 3.5 h. The solution was allowed to cool and then evaporated and the crude product purified by column chromatography over silica gel eluting with 4:1 hexanes/ethyl acetate to give the dinitrile **5**[4b] as colorless prisms (80 mg, 59%): mp = 126.2−128.2 °C (heptane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.73 (1H, ddd, J = 6.3, 3.3, 2.7 Hz), 3.36 (1H, dd, J = 6.3, 2.1 Hz), 2.76 (1H, dd, J = 17.2, 3.3 Hz), 2.74 (1H, m), 2.56 (1H, dd, J = 17.2, 8.3 Hz), 2.23 (2H, m), 1.64−1.96 (10H, m); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 117.7, 117.2, 76.1, 65.6, 61.8, 38.3, 35.8, 31.9, 29.4, 27.0, 23.1, 18.7, 17.4; IR (thin film, cm^{-1}) 2240, 1450; m/z (CI) 232 (M + H^+, 67%), 191 (100%). Anal. Calcd for $C_{13}H_{17}N_3O$: C, 67.51; H, 7.41; N, 18.17. Found: C, 67.57; H, 7.31; N, 18.05. **Crystal Data:** $C_{13}H_{17}N_3O$, M = 231.3. monoclinic, space group *Cc* (no. 9), a = 15.563(3), b = 9.938(2), c = 7.943(2) Å, β = 97.24(3) °, V = 1218.7(4) Å^3; Z = 4, D_c = 1.261 g/cm^3, $F(000)$ = 496, T = 140(1) K, μ(Mo Kα) = 0.8 cm^{-1}, λ(Mo Kα) = 0.71069 Å; 3096 reflections were measured, of which 2024 were unique (R_{int} = 0.078); 1946 were "observed" with $I > 2\sigma I$. Final R-factors: wR_2 = 0.159 and R_1 = 0.063 (1b) for all 2024 reflections weighted $w = [\sigma^2(F_o^2) + (0.128P)^2]^{-1}$ with $P = (F_o^2 + 2F_c^2)/3$; for the "observed" data only, R_1 = 0.062.

Conversion of Compound 14 to Compound 5. A solution of compound **14** (44 mg, 0.19 mmol) in anhydrous toluene (5 mL) was heated at 180 °C (oil bath temperature) in a sealed tube for 2.5 h. The solvent was evaporated and the product purified by column chromatography over silica gel eluting with 2:1 hexanes/ethyl acetate to give compound **5** as a white crystalline solid (42 mg, 95%).

Dialkene 3. A solution of dinitrile **5** (85 mg, 0.37 mmol) in toluene (5 mL) was cooled to −78 °C under an atmosphere of argon. DIBAL (0.5 mL of a 1.5 M solution in toluene) was added dropwise over 10 min and the reaction stirred at −78 °C for a further 2 h. Methanol (0.2 mL) was added and the reaction mixture allowed to warm to room temperature. An aqueous solution of potassium sodium tartrate (1.4 M, 5 mL) was added and the biphasic solution stirred vigorously for 2 h. The organic layer was separated and the aqueous layer extracted with ethyl acetate (10 mL). The combined organics were dried over sodium sulfate and evaporated to give dialdehyde **4** as a yellow oil (87 mg, 100%), which was used immediately. A solution of propyltriphenylphosphonium bromide (350 mg, 0.91 mmol) in THF (5 mL) was cooled to 0 °C under argon. Potassium hexamethyldisilazide (1.8 mL of a 0.5 M solution in toluene) was added and the solution stirred at 0 °C for 30 min. The solution was then cooled to −40 °C, and a solution of crude dialdehyde **4** (80 mg, 0.34 mmol) in THF (1 mL) was added dropwise. The solution was allowed to warm to room temperature over 24 h. A saturated aqueous solution of ammonium chloride (5 mL) and ethyl acetate (5 mL) were added, and the organic layer was separated, dried over sodium sulfate, and evaporated. The residue was purified by column chromatography eluting with 9:1 hexanes/ethyl acetate to give the colorless oil product as a mixture of alkene stereoisomers (60 mg, 62%). Data for major *Z,Z*′-stereoisomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.6 (1H, dt, J = 10.8, 7.2 Hz), 5.43−5.34 (3H, m), 5.28 (1H, dd, J = 10.8, 6.4 Hz), 3.45 (1H, dd, J = 9.3, 6.9 Hz), 2.62 (1H, m), 2.07−1.98 (6H, m), 1.70 (2H, m), 1.49 (2H, s, H-7), 1.35 (2H, m, H-11), 1.18 (4H, s, H-8 and H-9), 1.0 (2H, m), 0.93 (3H, t, J = 7.6 Hz), 0.88 (3H, t, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.25, 135.37, 124.82, 122.27, 77.04, 63.85, 51.56, 41.48, 33.16, 31.37, 28.68, 28.34, 20.95, 20.22, 19.71, 18.65, 17.03, 14.65, 13.40; IR (thin film, cm^{-1}) 1591, 1462, 1439; m/z (CI) 290.2 (M + H, 100%), 179 (28%), 220 (20%). HRMS (CI) Calcd for $C_{19}H_{31}NO$ (M + H): 290.2484. Found: 290.2482.

Perhydrohistrionicotoxin 2. To a solution of alkene **3** (5 mg, 0.017 mmol) in anhydrous methanol (5 mL) was added 10% palladium on charcoal (5 mg). The solution was stirred under an atmosphere of hydrogen for 48 h, after which time, the reaction mixture was filtered through Celite and concentrated. The crude product was purified by column chromatography over silica gel eluting with 95:5:0.5 dicloromethane/methanol/0.88 M aqueous ammonia solution to give perhydrohistrionicotoxin as a clear oil (3.5 mg, 69%). Spectroscopic data was in agreement with that previously reported.[4m]

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Supporting Information Available: X-ray crystal data for compounds **5** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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