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A Gram-Scale Batch and Flow Total Synthesis of Perhydrohistrionicotoxin

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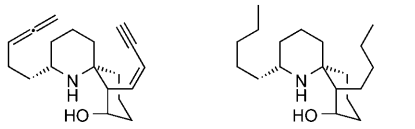
Abstract: The total synthesis of the spiropiperidine alkaloid (−)-perhydrohistrionicotoxin (perhydro-HTX) **2** has been accomplished on a gram scale by employing both conventional batch chemistry as well as microreactor techniques. (*S*)-(−)-6-Pentyltetrahydropyran-2-one **8** underwent nucleophilic ring opening to afford the alcohol **10**, which was elaborated to the nitrone **13**. Protection of the nitrone as the 1,3-adduct of styrene and side-chain extension to the unsaturated nitrile afforded a precursor **17**, which underwent dipolar cycloreversion and 1,3-dipolar cycloaddition to give the core spirocyclic precursor **18** that was converted into perhydro-HTX **2**. The principal steps to the spirocycle **18** have successfully been transferred into flow mode by using different types of microreactors and in a telescoped fashion, allowing for a more rapid access to the histrionicotoxins and their analogues by continuous processing.

Keywords: alkaloids · cycloaddition · flow chemistry · olefination · total synthesis

Introduction

The recognition of microreactor technology as a competitive way of processing chemical intermediates has been an important development in recent years, and synthesis under continuous flow conditions in many cases offers distinct advantages over conventional round-bottomed flask chemistry.[1] Microreactors enable the precise control of reaction parameters, linear scaleup and safe handling of hazardous substances with exquisite reproducibility. Many key chemical transformations transfer readily from batch into flow chemical procedures,[2] and flow chemistry can be a substantial support for diversity-oriented synthesis programs, and even has significant applications in the total synthesis of natural products and drug molecules.[3]

Herein, we describe the total synthesis of the reduced spiropiperidine alkaloid (−)-perhydrohistrionicotoxin (**2**), which has been achieved by a combination of conventional batch techniques and microreactor technology. The histrionicotoxins[4] constitute a class of poison-arrow frog alkaloids that have gained the considerable attention of chemists and biologists over almost 40 years; numerous approaches to these structurally challenging target molecules have been developed in the past.[5] We have contributed to this area by the use of intramolecular nitrone dipolar cycloaddition strategies and the continued improvement of practical procedures, leading to several general and highly competitive diastereoselective and enantioselective syntheses[6] of the family, including (−)-histrionicotoxin 285A (**1**) and **2**.



1 (−)-histrionicotoxin 285A **2** (−)-perhydrohistrionicotoxin

Our ongoing interest in the histrionicotoxins is fuelled by their biological activity, such as serving as non-competitive inhibitors of the neuromuscular, ganglionic and nicotinic acetylcholine receptors.[7] However, the full medicinal potential of the histrionicotoxins, or simplified analogues thereof, remains to be explored. From a synthetic point of view, two nitrone-based dipolar cycloaddition approaches appear to

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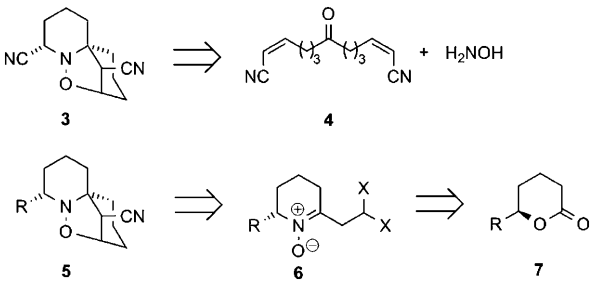
be the most suitable for the rapid access to diverse natural and non-natural members of the compound class (Scheme 1): Firstly, the domino cyclisation of bis-nitrile



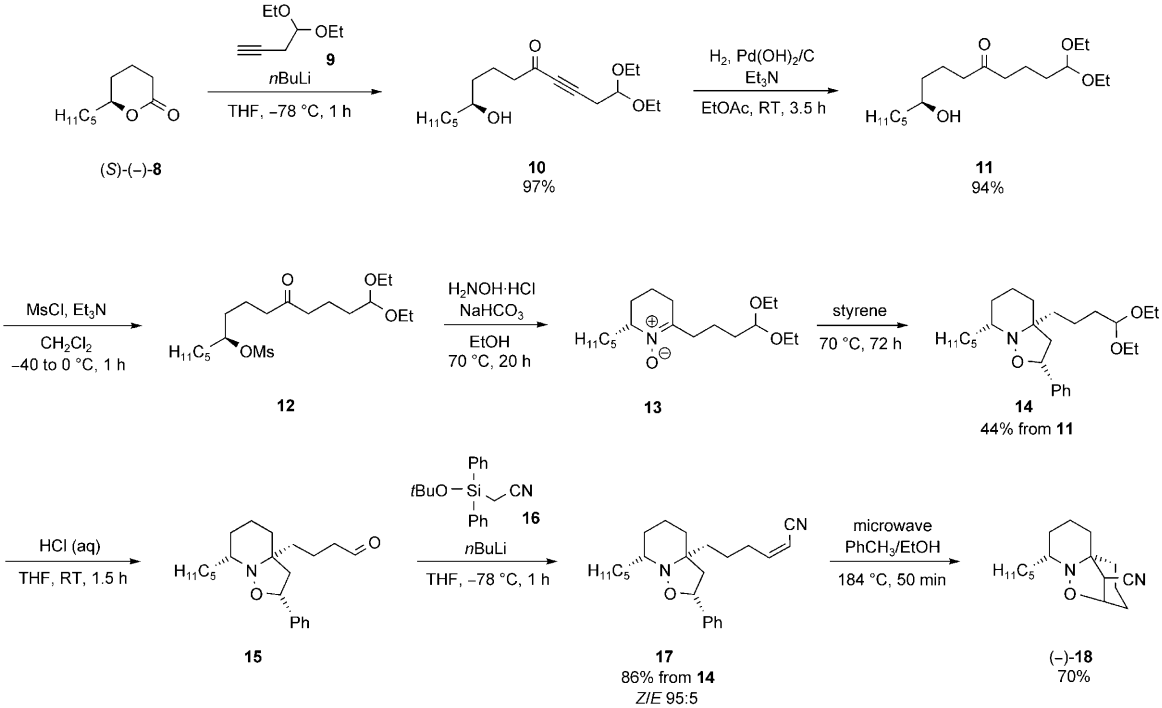
Scheme 1. Retrosynthetic analysis of spirocycles **3** and **5**.

ketone **4** with hydroxylamine, which elegantly leads to the racemic bifunctional 6,6,5-tricyclic bis-nitrile **3**,[8,6d] and secondly, the concise route to enantiomerically pure azaspirocycle **5** via cyclic nitrone **6**, which derives from a chiral δ-lactone **7**.[6e] The tricyclic isoxazolidines **3** and **5** are late-stage precursors to many members of the compound class of the histrionicotoxins, and they can also be regarded as ideal templates for the synthesis of novel analogues.

We have recently reported an improved batch synthesis of the ketone **4** and its conversion into isoxazolidine **3** under continuous flow conditions.[9] We further aimed at investigating a more generic route from chiral lactones such as **7** for flow processing based on a novel batch synthesis of **2**. This account describes a gram-scale batch synthesis of **2**, and demonstrates translation to continuous flow conditions.

Results and Discussion

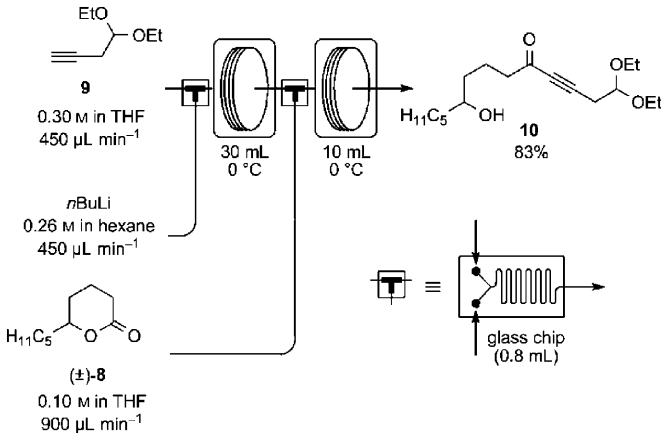
For the gram-scale synthesis of **2**, we required a substantial quantity of the *n*-pentyl-substituted 6,6,5-tricyclic isoxazolidine intermediate **18** (Scheme 2). While our previous approach to a related hydroxymethyl derivative involved a lengthy elaboration of (*S*)-(−)-glycidol,[6e] we consequently employed (*S*)-(−)-6-pentyltetrahydropyran-2-one (**8**) as the chiral starting material.[10] Nucleophilic addition of the lithium acetylide derived from alkyne **9**[11] to the lactone **8** at −78 °C provided the propargyl ketone product **10** in near quantitative yield. The alkyne **10** was hydrogenated over palladium(II) hydroxide on carbon in the presence of triethylamine to afford the saturated derivative **11**. Sulfonylation of the alcohol gave the mesylate **12**, which was converted into the nitrone **13** by formation of the oxime using an excess of hydroxylammonium chloride and sodium bicarbonate at 70 °C in ethanol, followed by intramolecular nucleophilic displacement of the sulfonate. Nitrone **13** was difficult to obtain in pure form, and therefore, it was immediately processed further by protection as its styrene adduct **14**. The 1,3-dipolar cycloaddition of nitrone **13** with styrene proceeded with useful diastereoselectivity to provide the separable *exo* isomer of isoxazolidine **14** in 44% over three steps from alcohol **11**. Acidic hydrolysis of acetal **14** led to aldehyde **15** and Peterson olefination of this intermediate by using Kojima's reagent **16**[12] gave α,β-unsaturated nitrile **17**



Scheme 2. Batch synthesis of 6,6,5-tricyclic isoxazolidine (−)-**18**. MsCl = mesyl chloride.

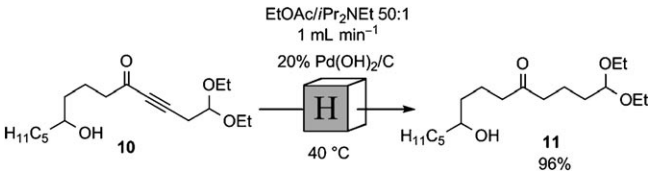
with excellent *Z* selectivity (*Z*/*E* 95:5). Finally, microwave heating of nitrile **17** at 184 °C led to loss of styrene by 1,3-dipolar cycloreversion and subsequent intramolecular 1,3-dipolar cycloaddition to provide the enantiopure 6,6,5-tricyclic nitrile (−)-**18** in 70 % yield.

As it stands, the route to isoxazolidine **18** represents an efficient and competitive means of obtaining this late-stage intermediate to **2** and its analogues, and it could be prepared enantiomerically pure in quantities in excess of two grams. However, we were interested in further simplification of processing, with particular emphasis on shortening of reaction times and reducing the number of purification steps, and we envisaged realising this objective using flow-chemistry techniques. The first challenge was the use of organometallic reagents under continuous flow conditions, and the timely addition of reagent volumes in telescoped flow chemical procedures. As shown in Scheme 3, the investigation started with the nucleophilic addition of lithiated alkyne **9** to racemic δ-lactone **8**, and after careful investigation of the reaction parameters, it could be performed in a telescoped three-channel flow chemical procedure at 0 °C, as an alternative to the batch reaction at −78 °C. As a process configuration, we used two piston pumps compatible with corrosive fluids (Ismatec)[13] in combination with a standalone HPLC pump (Knauer),[14] the mixing of the individual reaction channels were performed on glass chips (Sigma Aldrich).[15] A stock solution of the alkyne **9** in tetrahydrofuran was mixed with a stock solution of *n*-butyllithium in hexane, on a first glass chip, and the resulting flow stream was directed into a reactor coil made out of PTFE tubing (internal volume 30 mL, 1/8 in. o.d., 1.5 mm i.d.), where complete deprotonation occurred within a retention time of roughly 30 min. As soon as a constant flow stream of the lithiated alkyne emerged from the first reactor coil, a stock solution of the lactone (±)-**8** in tetrahydrofuran was channelled in on a second glass chip positioned after the first coil, and the resulting flow stream was passed through a second reactor coil (internal volume 10 mL), and into an aqueous solution of ammonium chloride at the reactor outlet. Extractive workup and drying in vacuum provided the propargyl ketone **10** in 83 % yield, and in a purity of 85–90 %, as estimated by ^{1}H NMR spectroscopy. As an alternative to the aqueous workup, an Omnifit-cartridge[16] containing QuadraPure-IDA dicarboxylic acid resin[17] could be connected to the reactor outlet, leading to a quench of the lithium alkoxide on the polymeric reagent. A noticeable feature of this telescoped flow procedure was the surprisingly long reaction time of around 30 min required for the deprotonation of the terminal alkyne **9** with *n*-butyllithium at 0 °C, whereas the nucleophilic attack of the resulting lithium acetylide anion on lactone **8** at the same temperature proceeded to completion within five minutes or less.



Scheme 3. Telescoped ring opening of lactone **8** to give propargyl ketone **10**.

The flow procedure shown in Scheme 3 could also be performed under steady-state conditions, delivering the propargyl ketone product **10** on a gram scale and with sufficient purity to be used in the next stage. As shown in Scheme 4,

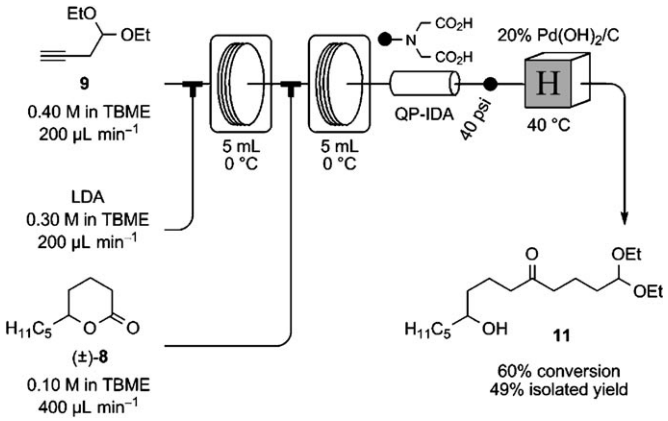


Scheme 4. Flow hydrogenation of alkyne **10** to the saturated alcohol **11**.

the crude alkyne **10** was subsequently subjected to flow hydrogenation in the H-cube hydrogenator (Thales Nano).[18] Hydrogenation using palladium(II) hydroxide on carbon as the catalyst at 40 °C in ethyl acetate containing Hünig's base, gave the saturated derivative **11** in near quantitative yield and in excellent purity. We have performed the hydrogenation of the alkyne **10** using starting material of varying purity. Under normal circumstances the alkyne **10** was contaminated with small amounts of the precursor alkyne **9**, which had been used in a slight excess in the previous step. While the alkyne **9** could be removed from the product **11** under high vacuum, it was more practical to hydrogenate the mixture of the alkynes **10** and **9** because the reduction product from **9** is more volatile and is easily removed upon concentration of the product solution under vacuum.

Since minor impurities in the alkyne **10** were unproblematic in the hydrogenation step, and the alcohol **11** was typically isolated in a pure state after workup, we envisaged combining the telescoped ring opening of lactone **8** and subsequent hydrogenation into a single flow process. To conduct experiments on a small scale of typically 0.2 to 0.5 mmol, we built a reactor system consisting of two Vapourtec R2+/R4 units;[19] the three reaction channels were controlled by two R2+ pump modules. The R2+ pump module enables the controlled small volume dosage of reagents through the in-built injection loops and by precise adjustment of the flow rates of the individual reaction channels, telescoped plug-flow synthesis involving several flow channels can be performed in a reliable and reproducible

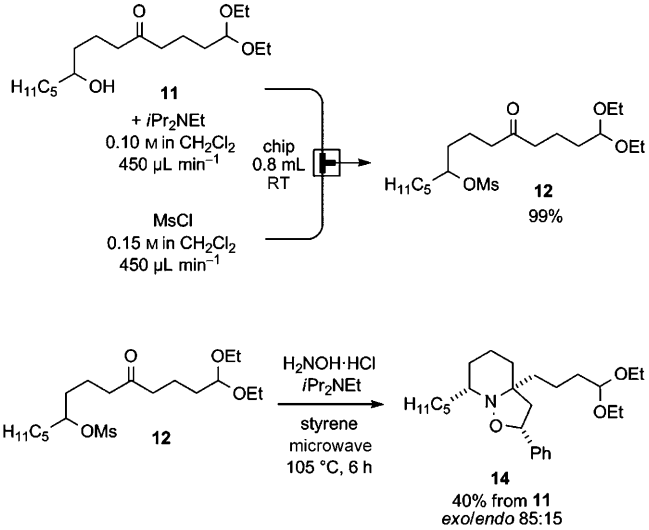
way. For the timely addition of reagents, we relied on the calculation of the residence times of the reagent volumes. As shown in Scheme 5, a solution of the alkyne **9** in *tert*-butyl methyl ether (TBME), and a solution of lithium diisopropylamide (LDA) in the same solvent were injected into sample loops of a Vapourtec R2+ unit. The two reagent channels were combined at ambient temperature in a Y-shaped PEEK mixer piece, and the resulting stream was directed into a 5 mL flow coil (PTFE tubing, 1/16 in. o.d., 1 mm i. d.) which was cooled to 0 °C. After the calculated residence time had passed, a solution of the lactone (±)-**8** in TBME was channelled in through a second Y piece, and the resulting stream was passed through a second 5 mL coil at 0 °C. The flow stream was passed through an Omnifit cartridge containing QuadraPure-IDA dicarboxylic acid resin, before entering the H-cube hydrogenator, where reduction took place over palladium(II) hydroxide on carbon at 40 °C. The flow stream was collected at the reactor outlet, to provide the saturated alcohol **11** as a mixture with the unreacted lactone **8**; conversion of the latter was 60 % by ^{1}H NMR spectroscopy. After chromatographic purification, the product **11** was isolated in a yield of 49 % (thus 82 % based on conversion of lactone **8**).



Scheme 5. Telescoped flow synthesis of alcohol **11**. QP-IDA = QuadraPure-IDA dicarboxylic acid resin.

By the described procedure we could demonstrate that the intermediate **11** could be produced in a single operation from starting materials **8** and **9**; however, the conversion of the lactone in the experiment shown in Scheme 5 was not complete. The use of an organometallic base such as LDA or *n*-butyllithium in such a process poses several problems.[20] When *n*-butyllithium in hexane was employed as the base in an analogous experiment, the conversion of the lactone **8** was increased to 80 %. However, reactor clogging by hydrolysis of the base to lithium hydroxide occurred rapidly, despite all precautions for the exclusion of moisture, making such a procedure impractical. On the other hand, LDA in TBME was much easier to handle, and extensive hydrolysis was avoided, but the diminished conversion (to 60 %) of lactone **8** was most likely attributed to the competition between 1,2-addition of the lithiated alkyne to the lactone and the enolisation of the carbonyl group by the residual diisopropylamine. At the same time, the presence of free diisopropylamine is beneficial in the terminal hydrogenation step, where an amine base is required to avoid the formation of decomposition or over-reduction products during the reduction of the intermediate alkyne **10** over the palladium catalyst.[6e]

The two-step flow preparation of alcohol **11** shown in Schemes 3 and 4 was the preferred method to obtain this intermediate on a preparative scale, and as shown in Scheme 6, alcohol **11** was subsequently elaborated into the isoxazolidine **14** by flow and batch microwave processing. A stock solution of the alcohol **11** and Hünig's base in dichloromethane was mixed on a glass chip with a solution of MsCl in the same solvent, at ambient temperature. Within only one minute, conversion of the alcohol was complete and a non-aqueous workup by filtration over basic alumina provided the pure mesylate **12** without any residual MsCl as a contaminant. The mesylate **12** was then converted into styrene adduct **14**, via the oxime and nitrone, in a convenient and straightforward one-pot microwave reaction. The substrate **12** was mixed with an excess of hydroxylammonium chloride and Hünig's base in neat styrene, and the mixture was heated in a microwave vial to 105 °C for 6 h. By way of this much simplified one-pot procedure, the reaction time for the conversion of mesylate **12** into isoxazolidine **14** could be significantly reduced, and workup of the sensitive intermediate nitrone **13** could be avoided. Inside a 20 mL microwave vial, we could process 1 mmol of substrate at a time, and we did not further attempt to develop a flow chemical procedure for this transformation. Isoxazolidine **14** was isolated in a yield of 40 %, as a mixture of the *exo* and *endo* isomer (ratio 85:15).[21]

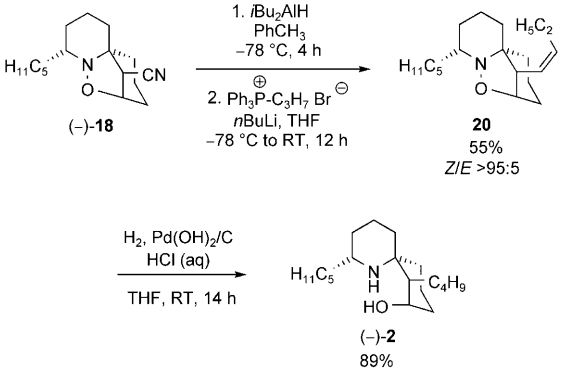


Scheme 6. Elaboration of alcohol **11** to isoxazolidine **14**.

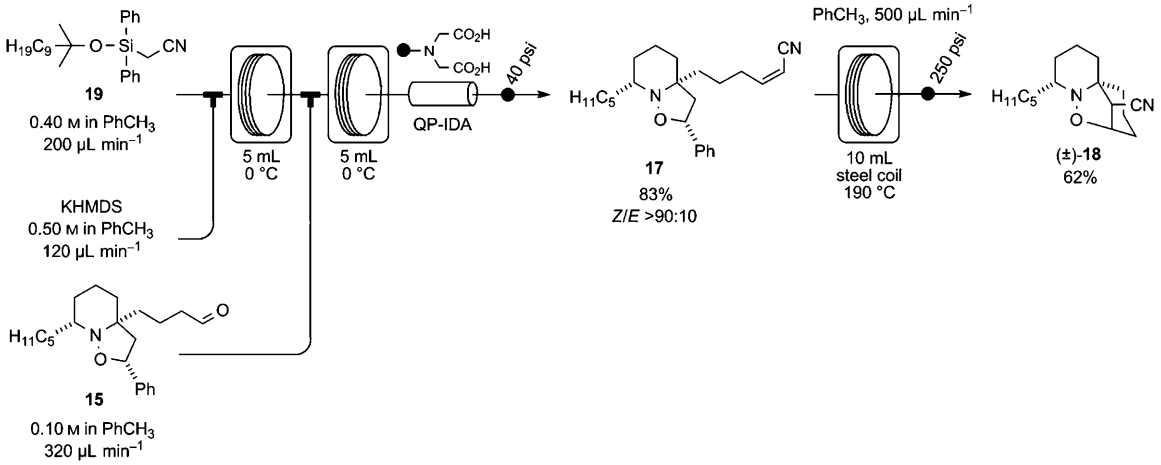
Next, we turned our attention to the Peterson olefination of aldehyde **15** with Kojima's reagent **16**[12] to prepare the α,β-unsaturated nitrile **17** under flow conditions. When reagent **16** was deprotonated with *n*-butyllithium in a flask, and the solution of the lithio-anion in tetrahydrofuran was mixed on a chip with a solution of the aldehyde **15** in the same solvent at 0 °C, we observed a complete conversion into nitrile **17** within just two minutes to give the same *Z*/*E* selectivity as that observed in the batch reaction at −78 °C. To telescope the deprotonation of silylacetonitile reagent **16** and the olefination of aldehyde **15**, we again used the reactor configuration shown in Scheme 5, and investigated the process on a small scale. Since we had previously encountered difficulties using *n*-butyllithium, we changed the solvent to toluene and used potassium bis-trimethylsilylamide (KHMDS) as the base. Gratifyingly, these conditions led to a comparable result and the nitrile **17** was obtained with >90:10 ratio of *Z*/*E* isomers (estimated by ^{1}H NMR spectroscopy), demonstrating that neither the counter cation nor the solvent have a significant effect on the stereochemical course of the reaction.

In the batch synthesis, we found the purification of α,β-unsaturated nitrile **17** was quite tedious, because the siloxane side products generated in the Peterson reaction as well as any excess of reagent **16** show a very similar R_f value to the product **17** on silica gel. To avoid an additional chromatography step in our flow synthesis, we therefore aimed to modify reagent **16** in a way that would enable more convenient removal of the silyl byproducts of the reaction. Consequently, we prepared silylacetonitrile reagent **19**, with a longer alkyl chain, by a procedure analogous to the preparation of reagent **16**,[12] using 2-methylundecan-2-ol[22] instead of *tert*-butanol. The telescoped Peterson olefination using this novel silylacetonitrile reagent is shown in Scheme 7. A flow stream of reagent **19** in toluene was mixed in a Y piece with a stock solution of KHMDS in toluene, at ambient temperature. The resulting flow stream was directed into a 5 mL coil cooled to 0 °C, and thereafter, a solution of aldehyde **15** in toluene was channelled in through a second Y piece. After the combined flow stream had passed through a second reactor coil at 0 °C, a quench was performed by using QuadraPure-IDA dicarboxylic acid resin. The crude product was collected, and charged onto a short plug of silica gel. This was first flushed with dichloromethane, which exclusively removed all residual reagent **19** and the siloxane byproducts. Thereafter, flushing with ethyl acetate gave the highly pure olefination product **17** in 83% yield, in a *Z*/*E* ratio of >90:10 by ^{1}H NMR spectroscopy. The subsequent thermal cycloreversion–cycloaddition of nitrile **17** to tricyclic isoxazolidine **18** proceeded readily under flow conditions, at 190 °C in a steel reactor coil, within a reaction time of 20 min, and a final column chromatography provided the separable (racemic) product (±)-**18** in 62% yield.

We completed the total synthesis of **2** by batch processing, within three further steps, as shown in Scheme 8. The final stages of the conversion of the polycyclic isoxazolidine **18** followed the strategy developed for a related molecule.[6e] Reduction with diisobutylaluminium hydride and Wittig olefination of the intermediate aldehyde with the ylide derived from propyltriphenylphosphonium bromide afforded the tri-



Scheme 8. Conversion of tricyclic isoxazolidine **18** into (−)-perhydrohistrionicotoxin **2**.



Scheme 7. Telescoped Peterson olefination of aldehyde **15** with the modified silylacetonitrile reagent **19** and thermal 1,3-dipolar cycloreversion–cycloaddition of isoxazolidine **17** to 6,6,5-tricyclic isoxazolidine (±)-**18**.

cyclic alkene **20**, which underwent concomitant N,O-bond cleavage and double-bond reduction upon hydrogenation over palladium(II) hydroxide on carbon. Enantiomerically pure **2**[23] was obtained from the batch synthesis in a quantity of one gram, and the ease of its preparation from isoxazolidine **18** implicates once more the potential utility of this template for the rapid production of analogues.

Conclusion

The overall sequence starting from an enantiopure δ-lactone **8** and the alkyne **9** leading to the 6,6,5-tricyclic isoxazolidine **18**, and subsequently, to the enantiopure perhydrohistrionicotoxin spiropiperidine structure represents one of the most efficient means of synthesising these challenging and biologically valuable target molecules today. We have demonstrated the effective use of microreactors and have succeeded in supporting the synthesis program by way of a multi-step flow synthesis of key building block isoxazolidine **18**, in racemic fashion.

The main chemical transformations of the batch synthesis of **2** could be linearly transferred into flow mode, thus establishing procedures that potentially allow for the production of multi-gram quantities of chemical intermediates by continuous processing, and also at real convenience for the experimentalist. The synthetic intermediates produced in flow showed comparable purity to those synthesised in the conventional way. The improved seven-step sequence towards isoxazolidine **18** involved only two chromatographic separations, and most products could be isolated in sufficiently pure condition by simple filtration through a minimal amount of silica or alumina adsorbants; aqueous workup was unnecessary in all cases, except the aqueous hydrolysis of acetal **14** to aldehyde **15**. Moreover, we demonstrated that flow processing, when involving the use of strong organometallic bases such as *n*-butyllithium, LDA and KHMDS can be performed with equal efficiency as batch processing, but at temperatures in the vicinity of 0 °C or higher, rather than "typical" −78 °C.[20] The example of a Peterson olefination performed at 0 °C in flow, which proceeds with comparable stereoselectivity to the batch reaction in a dry-ice bath, is a powerful demonstration of the advantage of flow processing.

Experimental Section

General methods: Batch reactions were performed under exclusion of air and moisture, in flame-dried glassware and under an atmosphere of nitrogen or argon. Flow reactions were performed with microreactor systems as specified in the respective experimental procedures. Microwave reactions were carried out in sealed reaction vessels using a Biotage Initiator 2.0 instrument (400 W). Commercial reagents were used without further purification, and enantiopure **8** was generously provided by ZEON Corporation.[10] Anhydrous solvents were obtained by passing them through columns of activated alumina (tetrahydrofuran, dichloromethane, toluene) or purchased from commercial suppliers (*tert*-butyl methyl ether, hexanes, with a boiling range from 40–60 °C). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer at 400 and 100.6 MHz, respectively, or on a Bruker DRX500 spectrometer at 500 and 125.8 MHz, respectively, using solutions in $CDCl_3$. Chemical shifts are reported relative to the resonances of $CHCl_3$ at $\delta = 7.25$ ppm (H) and $\delta = 77.0$ ppm (C). Microanalyses were performed by Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were recorded on an Electrothermal IA9300 digital melting point apparatus and are uncorrected. Positive-ion EI mass spectra were run on a ThermoQuest MAT95XL mass spectrometer using an ionisation energy of 70 eV. Accurate mass measurements were obtained with a resolution of 5000–10000 using perfluorokerosene as the reference compound. High-resolution positive-ion electrospray mass spectra were acquired with a Micromass Q-TOF II mass spectrometer using a cone voltage of 50 V and a capillary voltage of 3.0 kV. The sample was introduced by direct infusion at a rate of 5 μL min^{-1} using PEG400 as an internal calibrant. Unless otherwise indicated, flash chromatography was carried out by using Merck Kieselgel 60 (230–400 mesh; particle size 0.04–0.63 mm) silica gel. "Silica gel funnel chromatography" was performed in an analogous fashion as flash chromatography except that a sintered glass funnel was employed (silica bed: width 8 cm, height 7 cm) and passage of solvents was accelerated through the silica bed by reduced rather than positive pressure. Analytical TLC was conducted on Sigma–Aldrich silica gel coated aluminium sheets and visualised with UV and/or by phosphomolybdic acid/EtOH or potassium permanganate reagents.

Batch synthesis of 6,6,5-tricyclic isoxazolidine (−)-18

(9*S*)-1,1-Diethoxy-9-hydroxy-tetradec-3-yne-5-one 10: A solution of 4,4-diethoxybut-1-yne **9**[11] (12.1 g, 85.4 mmol) in THF (250 mL) was cooled to −78 °C. *n*BuLi (53.7 mL, 1.59 M in hexane, 85.4 mmol) was added dropwise (10 min) and the pale yellow solution was stirred (30 min). A solution of the lactone (*S*)-(−)-**8**[10] (12.1 g, 71.2 mmol) in THF (100 mL) was added dropwise (15 min) and this solution was stirred for a further 1 h. A saturated aqueous solution of NH_4Cl (100 mL) was added and the mixture was allowed to warm to RT. This mixture was diluted with H_2O and EtOAc and the organic layer was separated. The aqueous layer was extracted (2×) with EtOAc and the combined organic layers were washed with saturated aqueous solutions of $NaHCO_3$ and NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel funnel chromatography (EtOAc/hexanes containing 1% Et_3N, gradient; 10:90, 15:85, 20:80 then 25:75) to yield **10** as a clear, colourless oil (21.6 g, 97%). R_f 0.39 (25:75 EtOAc/toluene); $[\alpha]_D^{25} = -1.5$ (*c* 1.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.86$ (t, $J = 6.9$ Hz, 3H), 1.20 (t, $J = 7.1$ Hz, 3H), 1.23–1.51 (m, 10H), 1.66–1.88 (m, 2H), 2.55 (t, $J = 7.2$ Hz, 2H), 2.68 (d, $J = 5.6$ Hz, 2H), 3.49–3.59 (m, 3H), 3.61–3.71 (m, 2H), 4.68 ppm (t, $J = 5.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$, 15.1, 20.0, 22.6, 25.2, 25.4, 31.8, 36.5, 37.4, 45.2, 62.1, 71.3, 81.7, 89.2, 100.0, 187.9 ppm; IR (neat) 3473, 2929, 2868, 2218, 1710, 1675, 1455, 1371, 1343, 1227, 1119, 1061 cm^{-1}; HRMS (ESI): *m*/*z* calcd for $C_{18}H_{32}O_4Na$: 335.2198 $[M+Na]^+$; found: 335.2203.

(9*S*)-1,1-Diethoxy-9-hydroxy-tetradecan-5-one (11): A mixture of the alkynone **10** (21.1 g, 67.6 mmol), $Pd(OH)_2$ on carbon (1.3 g of 20% Pd catalyst) and Et_3N (2.0 mL) in EtOAc (150 mL) was shaken vigorously under an atmosphere of H_2 (25 psi, 3.5 h). The mixture was filtered through Celite washing with EtOAc. The combined filtrate and washings were concentrated and the residue was purified by silica gel funnel chromatography (EtOAc/hexanes containing 1% Et_3N, gradient; 10:90, 15:85, 30:70 then 35:65) to yield alcohol **11** (20.7 g, 94%) as a colourless, waxy solid: R_f 0.20 (25:75 EtOAc/toluene); $[\alpha]_D^{26} = -4.5$ (*c* 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.18, (t, $J = 7.1$ Hz, 6H), 1.22–1.82 (m, 16H), 2.42 (t, $J = 7.0$ Hz, 4H), 3.42–3.67 (m, 4H), 4.46, (t, 5.2 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 15.3, 19.1, 19.7, 22.6, 25.3, 31.8, 33.0, 36.8, 37.4, 42.3, 42.5, 61.1, 71.4, 102.7, 211.0 ppm; IR (neat) 3291, 3203, 2922, 2869, 1701, 1456 cm^{-1}; HRMS (ESI) *m*/*z* 339.2526 $C_{18}H_{36}O_4Na$ $[M+Na]^+$ requires 339.2511.

(2*R*,6*R*,8*R*)-6-(4′,4′-Diethoxy-1′-butyl)-2-pentyl-8-phenyl-1-aza-9-oxabicyclo[4,3,0]no-nane (14): To a solution of the alcohol **11** (10.1 g, 32.1 mmol) and Et_3N (17.8 mL, 128 mmol) in CH_2Cl_2 (250 mL) at −40 °C was added MsCl (4.96 mL, 64.2 mmol) in CH_2Cl_2 (50 mL) dropwise

(5 min). This mixture was allowed to warm to 0 °C over 1 h. Saturated aqueous $NaHCO_3$ was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel funnel chromatography (EtOAc/hexanes containing 1% Et_3N, gradient; 5:95, 10:90, 15:85 then 25:75) to give the mesylate **12** (11.1 g) as a clear, colourless oil which was used immediately in the next step. Analytical data: R_f 0.28 (30:70 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J= 6.8 Hz, 3H), 1.18, (t, J=7.2 Hz, 6H), 1.22–1.44 (m, 6H), 1.53–1.73 (m, 10H) 2.39–2.46 (m, 4H), 2.99 (s, 3H), 3.47 (qd, J=7.1, 9.5 Hz, 2H), 3.62 (qd, J=7.1, 9.5 Hz, 2H), 4.46, (t, J=5.3 Hz, 1H), 4.69 (m_c, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.8, 15.2, 18.8, 18.9, 22.3, 24.5, 31.4, 32.9, 33.7, 34.2, 38.6, 41.8, 42.3, 61.0, 83.4, 102.6, 210.0 ppm. Mesylate **12** (11.1 g) was taken up in EtOH (250 mL) and $NaHCO_3$ (14.7 g, 174 mmol) and $NH_2OH{\cdot}HCl$ (11.7 g, 168 mmol) were added sequentially. This mixture was stirred at RT (30 min), then at 70 °C (20 h). The reaction mixture was allowed to cool to RT, H_2O (200 mL) was added and the mixture was concentrated to approximately 200 mL volume. The mixture was diluted with CH_2Cl_2 and the organic layer was separated. The aqueous layer was extracted (4 ×) with CH_2Cl_2 and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated to afford crude nitrone **13** (10.1 g) as a pale yellow oil which was used immediately in the next step without characterisation, R_f 0.34 (4:96 EtOH/CH_2Cl_2). Crude nitrone **13** (10.1 g) was taken up in freshly distilled styrene (200 mL) and heated at 70 °C (72 h). The mixture was allowed to cool to RT, concentrated and the residue was purified by flash chromatography (EtOAc/hexanes containing 1% Et_3N, gradient; 2:98, 4:96 then 6:94) to yield the isoxazolidine *exo*-**14** (5.89 g, 44%) as a colourless oil: R_f 0.33 (10:90 EtOAc/hexanes); $[\alpha]_D^{25}=-16.2$ (c 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J=6.8 Hz, 3H), 1.14 (t, J=6.8 Hz, 3H), 1.15 (t, J=6.8 Hz, 3H), 1.17–1.70 (m, 16H), 1.75–1.97 (m, 3H), 2.00 (dd, J=5.2, 12.5 Hz, 1H), 2.63, (t, J=11.1 Hz, 1H), 2.67–2.77 (m, 1H), 3.31–3.47 (m, 2H), 3.49–3.61 (m, 2H), 4.39, (t, J=5.6 Hz, 1H), 5.39, (dd, J=5.0, 10.0 Hz, 1H), 7.18–7.41 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 15.3, 18.9, 20.0, 22.6, 25.4, 29.5, 31.3, 32.1, 33.9, 34.6, 41.5, 42.2, 59.3, 60.7, 61.0, 67.8, 76.9, 102.6, 126.0, 127.0, 128.3, 142.0 ppm; IR (neat) 2929, 2865, 1450, 1374, 1125, 1063, 754, 698 cm^{-1}; $C_{38}H_{53}NO_4Si$ requires C, 74.10; H, 8.67; N, 2.27, found: C, 74.17; H, 8.63; N, 2.29; HRMS (EI) m/z 417.3220. $C_{26}H_{43}NO_3$ $[M]^{+\cdot}$ requires 417.3237.

(2*R*,6*S*,8*R*)-6-(5′-Cyanopent-4′-en-1′-yl)-2-pentyl-8-phenyl-1-aza-9-oxabicyclo[4,3,0]-nonane 17: To a solution of diethylacetal **14** (5.89 g, 14.1 mmol) in THF (100 mL) was added aqueous HCl (20.0 mL of a 2.0 M solution, 40.0 mmol). After 1.5 h, saturated aqueous $NaHCO_3$ (40 mL) was added. The mixture was further diluted with H_2O and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 ×) and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was filtered through a plug of silica, washing with EtOAc/hexanes (25:75). The filtrate and washings were concentrated to give the aldehyde **15** (5.55 g) as a colourless oil which was used immediately in the next step. Analytical data: R_f 0.41 (20:80 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (t, J=6.8 Hz, 3H), 1.07 (m_c, 1H), 1.14–1.80 (m, 16H), 1.85 (m_c, 1H), 1.90 (dd, J=5.2, 12.5 Hz, 1H), 2.23 (dddd, J=1.7, 6.3, 8.1, 17.4 Hz, 1H), 2.34 (dddd, J=1.7, 6.3, 8.1, 17.4 Hz, 1H), 2.62 (dd, J=10.3, 12.5 Hz, 1H), 2.66 (tt, J=3.2, 8.2 Hz, 1H), 5.33 (dd, J=5.2, 10.3 Hz, 1H), 7.19–7.14 (m, 1H), 7.23–7.33 (m, 4H), 9.60 (t, J=1.7 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 16.6, 19.9, 22.7, 25.4, 29.4, 31.0, 32.2, 34.5, 41.6, 42.2, 44.2, 59.3, 67.7, 76.8, 125.9, 127.1, 128.3, 141.9, 202.7 ppm. Meanwhile, to a solution of (tBuO)Ph_2SiCH_2CN[12] (5.20 g, 17.6 mmol) in THF (150 mL) at −78 °C was added nBuLi (11.0 mL, 1.60 M in hexanes, 17.6 mmol) dropwise (over ≈1 min). The mixture was allowed to warm to 0 °C, kept at this temperature for 10 min, and then cooled to −78 °C. To this mixture was added dropwise (over ≈10 min) the crude aldehyde (5.55 g) in THF (50 mL). After 1 h, saturated aqueous NH_4Cl (40 mL) was added and the mixture was allowed to warm to RT. The mixture was diluted with H_2O and EtOAc and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 ×) and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 3:97, 4:96, 5:95 then 6:94) to yield a 95:5 Z/E-mixture of α,β-unsaturated nitrile **17** (4.45 g, 86%) as a clear, colourless oil: R_f 0.27 (10:90 EtOAc/hexanes); $[\alpha]_D^{24}=-13.8$ (c 0.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) for major Z isomer δ 0.88 (t, J=6.8 Hz, 3H), 1.06–1.71 (m, 15H), 1.75–1.87 (m, 2H), 1.88–2.01 (m, 2H), 2.27–2.38 (m, 2H), 2.64–2.77 (m, 2H), 5.20 (d, J=10.9 Hz, 1H), 5.41 (dd, J= 5.0, 10.1 Hz, 1H), 6.31 (dt, J=7.6, 10.8 Hz, 1H), 7.21–7.41 (m, 5H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) for major (Z-) isomer δ 14.0, 19.9, 22.6, 25.3, 29.5, 31.0, 31.9, 32.1, 34.5, 41.3, 42.1, 59.4, 67.6, 76.7, 99.4, 116.0, 125.9, 127.0, 128.3, 142.0, 155.0 ppm; IR (neat) 2929, 2860, 2219, 1453, 753, 698 cm^{-1}; HRMS (EI) m/z 366.2660. $C_{24}H_{34}N_2O$ $[M]^{+\cdot}$ requires 366.2666. Characteristic signals for minor E isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.24 (dt, J=1.6, 16.4 Hz, 1H); 6.58 (td, J=6.9, 16.4 Hz, 1H), ppm.

(1*R*,5*R*,8*S*,12*R*)-12-Cyano-5-pentyl-6-aza-7-oxatricyclo[6.3.1.01,6]dodecane (−)-18: A solution of the (95:5, Z/E) α,β-unsaturated nitrile **17** (4.03 g, 11.0 mmol) in $PhCH_3$ (114 mL) and EtOH (19 mL) was divided equally into 19 vials. These vials were each sealed and irradiated in a microwave reactor for 50 min at 184 °C (absorption level "normal", the pressure was 11 bar). The contents of the vials were combined, concentrated and the residue was purified by flash chromatography (EtOAc/hexanes, gradient; 3:97, 4:96 then 5:95) to yield the tricycle **18** (2.03 g, 70%) as a colourless oil: R_f 0.23 (8:92 EtOAc/hexanes) $[\alpha]_D^{24}=-201.9$ (c 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J=6.8 Hz, 3H), 1.06–1.40 (m, 9H), 1.50–1.70 (m, 4H), 1.71–2.04 (m, 6H), 2.11–2.20 (m, 1H), 2.33–2.43 (m, 1H), 3.43 (d, J=5.5 Hz, 1H), 4.64–4.75 (m, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 17.5, 19.2, 22.6, 25.3, 27.1, 29.6, 32.0, 32.2, 34.1, 36.0, 38.1, 65.5, 65.5, 75.6, 117.9 ppm; IR (KBr disc) 2939, 2861, 2236, 1456, 1082, 930 cm^{-1}; HRMS (EI) m/z 262.2035. $C_{16}H_{26}N_2O$ $[M]^{+\cdot}$ requires 262.2040.

Flow- and microwave-assisted synthesis of 6,6,5-tricyclic isoxazolidine (±)-18

Telescoped flow preparation of propargyl ketone 10: A solution of alkyne **9** (4.26 g, 30.0 mmol) in THF was diluted to 100 mL in a volumetric flask (resulting in a 0.30 M solution). Likewise, a solution of nBuLi (16.3 mL, 1.60 M in hexane, 26.1 mmol) in hexane was diluted to 100 mL in a volumetric flask (resulting in a 0.26 M solution), and a solution of lactone (±)-**8** (0.43 g, 2.53 mmol) in THF, diluted to 25 mL in a volumetric flask (resulting in a 0.10 M solution). The stock solutions of nBuLi and alkyne **9** were pumped (piston pumps, Ismatec[13]) at a rate of 450 μL min^{-1} each, onto a glass chip (internal volume 0.8 mL, Sigma Aldrich[15]), where mixing occurred at 0 °C. The resulting flow stream was directed into a 30 mL reactor coil (PTFE tubing, 1/8 in. o.d., 1.5 mm i.d.), cooled to 0 °C, then onto a second identical glass chip. As soon as a constant flow stream of the lithiated alkyne entered the second glass chip, evident by residence time calculation and the light yellow colour of the solution, it was combined at 0 °C with a flow stream of lactone (±)-**8** (pumped at 900 μL min^{-1} with a Knauer HPLC pump[14]). The resulting flow stream was passed through a second reactor coil (10 mL), cooled to 0 °C and into an aqueous solution of NH_4Cl at the reactor outlet. After the stock solution of the lactone had been consumed, rinse was performed using THF until all reagent had exited the reactor (residual stock solutions of alkyne and nBuLi were left unreacted). The crude product mixture was transferred into a separation funnel, the layers were separated and the aqueous layer was extracted with EtOAc (3 ×). The combined organic layers were dried ($MgSO_4$), filtered, evaporated and drying in vacuum afforded ketone **10** (0.65 g, 83%) as a light yellow oil, purity 85–90% as estimated by ^{1}H NMR spectroscopy, the product being spectroscopically identical to the product obtained by the batch procedure.

Flow hydrogenation of propargyl ketone 10 to alcohol 11: Propargyl ketone **10** (386 mg containing ≈10% of alkyne **9** by ^{1}H NMR spectroscopy, ≈1.17 mmol) was dissolved in EtOAc (45 mL) and iPr$_2$NEt (0.90 mL) and the solution was passed through the H-cube hydrogenator [Thales Nano,[18] catalyst 20% $Pd(OH)_2$/C, mode "full H_2"] at 1 mL min^{-1}, the catalyst being heated at 40 °C. The product mixture was collected at the reactor outlet, evaporated and vacuum-dried to afford pure alcohol **11**

(354 mg, 96%) as a colourless oil, spectroscopically identical to the product obtained by the batch procedure.

Flow mesylation of alcohol 11 to mesylate 12: MsCl (0.12 mL, 1.55 mmol) was diluted to a 10 mL volume with CH_2Cl_2 in a volumetric flask. Likewise, a CH_2Cl_2 solution of alcohol **11** (316 mg, 1.00 mmol) and iPr$_2$NEt (0.35 mL, 2.00 mmol) was diluted to a 10 mL volume in a second volumetric flask. The two stock solutions were pumped (piston pumps, Ismatec[13]) at a rate of 450 μL min^{-1} each, onto a glass chip (internal volume 0.8 mL, Sigma Aldrich[15]), where mixing occurred at RT. After the stock solutions had been consumed, rinse was performed with CH_2Cl_2 until all reagents had exited the reactor. The product mixture was collected at the reactor outlet and evaporated onto basic alumina. The crude product was charged onto a short plug of basic alumina and flushed with Et_2O. The filtrate was concentrated and dried in vacuum to afford pure mesylate **12** (390 mg, 99%) as a colourless oil, spectroscopically identical to the product obtained by the batch procedure.

One-pot microwave reaction of mesylate 12 to isoxazolidine 14: A mixture of mesylate **12** (395 mg, 1.00 mmol), iPr$_2$NEt (2.10 mL, 12.00 mmol) and $H_2NOH{\cdot}HCl$ (556 mg, 8.00 mmol) in styrene (11 mL) was stirred in a 20 mL microwave vial until homogeneous (≈15 min), then heated in the microwave at 105 °C for 6 h (absorption level "high"). The mixture was filtered through a plug of basic alumina with the aid of EtOAc, concentrated and vacuum-dried. The residue was purified by chromatography (EtOAc/hexanes 1:8 containing 1% iPr$_2$NEt) to afford isoxazolidine **14** (166 mg, 40%) as a 85:15 mixture of *exo* and *endo* isomers, the major (separable) component being spectroscopically identical to the product obtained by the batch procedure described above. The minor component, the *endo* isomer,[21] could not be obtained in pure form. Its characteristic NMR signals were obtained from a mixture fraction: *endo*-**14**: R_f 0.40 (10:90 EtOAc/hexanes); characteristic signals: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.53, (t, J = 5.6 Hz, 1H), 5.23, (t, J = 8.7 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 15.4, 19.3, 19.5, 22.7, 25.0, 29.68, 29.74, 30.9, 34.2, 34.3, 40.3, 40.6, 60.9, 61.3, 62.5, 67.6, 79.9, 102.8, 125.2, 126.7, 128.1, 144.6 ppm.

Preparation of silyl reagent 19, 2-[(2-methylundecan-2-yloxy)diphenylsilyl]acetonitrile: Ph_2SiCl_2 (1.79 mL, 8.51 mmol) was added at RT to a mixture of 2-methylundecan-2-ol[22] (1.58 g, 8.50 mmol) and iPr$_2$NEt (1.53 mL, 8.78 mmol) in CH_2Cl_2 (25 mL) and the mixture was stirred at reflux for 4 days. The mixture was concentrated to dryness and the residue was taken up in petroleum ether (100 mL) and stirred for 15 min. The mixture was filtered through Celite with the aid of petroleum ether and the filtrate was concentrated and vacuum dried to afford the intermediate monochlorosilane as a colourless oil (2.11 g, 62%), which was used immediately in the next step without characterisation. nBuLi (6.55 mL, 1.60 M in hexane, 10.48 mmol) was added at 0 °C to a solution of iPr$_2$NH (1.52 mL, 10.84 mmol) in THF (12 mL) and the mixture was stirred for 30 min. Dry MeCN (0.29 mL, 5.55 mmol) was added and the mixture was stirred at 0 °C for 1 h. A solution of the chlorosilane (2.11 g, 5.23 mmol) in THF (5 mL) was added and the mixture was stirred at RT for 2 h. After addition of an aqueous solution of NH_4Cl (20 mL), the layers were separated and the aqueous layer was extracted with Et_2O (3×). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and purified by chromatography (petroleum ether/CH_2Cl_2 1:1) to give the silyl reagent **19** as a colourless oil (1.65 g, 80%). R_f 0.55 (50:50 petroleum ether/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.89 (t, J = 6.8 Hz, 3H), 1.17–1.42 (m, 20H), 1.46–1.51 (m, 2H), 2.14 (s, 2H), 7.38–7.50 (m, 6H), 7.62–7.66 ppm (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 5.7, 14.1, 22.7, 24.4, 29.3, 29.56, 29.58, 29.8, 30.0, 31.9, 44.6, 77.3, 118.4, 128.1, 130.6, 133.8, 134.6 ppm; IR (thin film) 2924, 2853, 2236, 1466, 1428, 1384, 1367, 1146, 1112, 1046, 1027 cm^{-1}; HRMS (EI) m/z calcd for $C_{26}H_{37}NOSi$: 407.2644 $[M]^+$; found: 407.2629.

Telescoped Peterson olefination of aldehyde 15 with silyl reagent 19 to nitrile 17: Two Vapourtec R2+/R4 units[19] were connected as shown in Scheme 5 (also see Figure S2 in the Supporting Information) and two 5 mL reactor coils (PTFE tubing, 1/16 in. o.d., 1 mm i. d.), separated by a one-way check valve, were appended to the system, as well as an omnifit column (150 mm, 10 mm bore)[16] filled with QP-IDA (5.50 g, 0.16 mmol g^{-1}). The system was primed with $PhCH_3$. Three reagent solutions of 2 mL volume each were injected into the sample loops connected to three HPLC pumps. A solution of KHMDS (0.50 M in $PhCH_3$, 2 mL) was pumped at a rate of 120 μL min^{-1} into a Y-shaped mixer piece (PEEK), where it was combined with a solution of silyl reagent **19** (305 mg, 0.78 mmol in 2 mL $PhCH_3$, 0.39 M), pumped at a rate of 200 μL min^{-1}. The resulting flow stream was directed into a 5 mL reactor coil, cooled to 0 °C, and into a second Y piece thereafter, connected to a second 5 mL reactor coil. After the calculated residence time of the deprotonated reagent **19** in the first reactor coil had passed, a solution of aldehyde **15** (68.0 mg, 0.20 mmol in 2 mL $PhCH_3$, 0.10 M) was channelled in through the second Y piece, and the resulting flow stream passed through the second 5 mL reactor coil at 0 °C. As the aldehyde solution was consumed (by time calculation), the first two reaction channels were switched off, and the third pump was set to 320 μL min^{-1}, and rinse was performed until all material had exited the reactor through the omnifit cartridge. The crude product was collected, evaporated onto silica and charged onto a short plug of silica. This was flushed with CH_2Cl_2 (100 mL), the filtrate was discarded, then with EtOAc (100 mL). The EtOAc flushings were evaporated and dried in vacuum to provide pure nitrile **17** as a light yellow oil (61.0 mg, 83%), showing a Z/E ratio of >90:10 by ^{1}H NMR spectroscopy and was spectroscopically identical to the product obtained by the batch procedure.

Flow rearrangement of nitrile 17 to isoxazolidine (±)-18: A solution of nitrile **17** (61.0 mg, 166 μmol) in $PhCH_3$ (2 mL) was injected into a 2 mL sample loop (Vapourtec R2+/R4[19]), and passed through a steel reactor coil (10 mL) at 500 μL min^{-1} heated to 190 °C. The product mixture was collected at the reactor outlet, evaporated and purified by chromatography (EtOAc/hexanes 1:10) to afford isoxazolidine (±)-**18** as a colourless oil (27.0 mg, 62%), spectroscopically identical to the product obtained by the batch procedure.

Conversion of 6,6,5-tricyclic isoxazolidine (−)-18 into 2 (1*R*,5*R*,8*S*,12*S*)-(1′*Z*)-12-(But-1′-enyl)-5-pentyl-6-aza-7-oxatricyclo[6.3.1.01,6]dodecane 20: iBu$_2$AlH (9.14 mL, 1.50 M in $PhCH_3$, 13.7 mmol) was added to a solution of the nitrile (−)-**18** (1.80 g, 6.85 mmol) in $PhCH_3$ (50 mL) at −78 °C. After 4 h, MeOH (1 mL) was added and the mixture was allowed to warm to RT. The mixture was diluted with EtOAc (50 mL) and aqueous potassium sodium tartrate (1.4 M, 100 mL) and stirred vigorously (4 h). The organic layer was separated and the aqueous layer was extracted with EtOAc (2×) and the combined organic layers were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was filtered through a plug of silica, washing with EtOAc/hexanes (25:75). The filtrate and washings were concentrated to give the intermediate aldehyde (1.85 g) as a colourless oil [R_f 0.37 (20:80 EtOAc/hexanes)] that was used immediately. Meanwhile, to a mixture of propyltriphenylphosphonium bromide (3.96 g, 10.3 mmol) in THF (20 mL) at −78 °C was added nBuLi (6.47 mL, 1.59 M in hexanes, 10.3 mmol) dropwise (over ≈3 min). The light yellow mixture was allowed to warm to 0 °C and stirred for 30 min whereupon the mixture had turned orange. The mixture was cooled to −78 °C and the crude aldehyde (1.85 g) in THF (30 mL) was added dropwise (over ≈20 min). This mixture was allowed to slowly warm to RT (over ≈4 h) and stirred overnight. A saturated aqueous solution of NH_4Cl (20 mL) was added and the mixture was diluted with H_2O and EtOAc and the organic layer was separated. The aqueous layer was extracted (2×) with EtOAc and the combined organic layers were washed sequentially with saturated aqueous solutions of $NaHCO_3$ and NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes; 2:98, 4:96 then 5:95) to yield the Z-alkene **20** as a clear, colourless oil (1.11 g, 55%). R_f 0.29 (8:92 EtOAc/hexanes); $[\alpha]_D^{24} = -160.8$ (c 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.86 (t, J = 6.5 Hz, 3H), 0.98 (t, J = 7.5 Hz, 3H), 1.04–1.63 (m, 16H), 1.7–1.82 (m, 2H), 1.85–2.02 (m, 2H), 2.04–2.18 (m, 2H), 2.56–2.68 (m, 1H), 3.42 (dd, J ≈ 7.2 Hz, 1H), 4.32–4.39 (m, 1H), 5.42 (t, J = 10.5 Hz, 1H), 5.62 ppm (dt, J = 11.0, 7.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.0, 14.4, 17.9, 19.6, 21.2, 22.6, 25.0, 25.7, 29.8, 32.2, 32.3, 34.2, 34.7, 42.5, 64.8, 64.8, 77.9, 123.3, 136.2 ppm; IR (thin film): 3022, 2928, 2858, 1459, 926 cm^{-1}; HRMS (EI): m/z calcd for $C_{19}H_{33}NO$: 291.2557 $[M]^+$; found: 291.2557.

(−)-Perhydrohistrionicotoxin 2: A mixture of the alkene **20** (1.05 g, 3.61 mmol), aqueous HCl (8.00 mL, 1.00 M, 8.00 mmol) and $Pd(OH)_2$ on carbon (200 mg of 20% Pd catalyst) in THF (150 mL) was shaken vigorously under an atmosphere of H_2 (25 psi, 14 h). The mixture was filtered through Celite, washed with THF and then THF/H_2O (1:1). The combined filtrate and washings were concentrated and the residue was diluted with a saturated aqueous solution of $NaHCO_3$ and EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×). The combined organic layers were washed with a saturated aqueous solution of NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes; 30:70 then MeOH/CH_2Cl_2/aqueous NH_3 (14 M); 2:98:0, 2:97:1, 5:94:1 then 10:89:1) to afford **2** as a waxy colourless solid (953 mg, 89%). R_f 0.28 (10:90 MeOH/CH_2Cl_2); $[\alpha]_D^{21} = -64$ (*c* 0.97, $CHCl_3$; lit. [24] −84.1);[23] ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 0.76$ (td, $J = 13.8$, 3.8 Hz, 1H), 0.86 (t, $J = 7.2$ Hz, 3H), 0.90 (t, $J = 6.8$ Hz, 3H), 1.04–1.17 (m, 2H), 1.20–1.43 (m, 15H), 1.44–1.66 (m, 5H), 1.73 (m, 1H), 1.81 (dt, $J = 13.8$, 2.5 Hz, 1H), 2.03 (qt, $J = 13.8$, 4.2 Hz, 1H), 2.20 (d, $J = 9.7$ Hz, 1H), 2.92 (m, 1H), 3.92 ppm (d, $J = 2.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$, 14.1, 15.2, 19.5, 22.5, 23.0, 25.6, 27.6, 27.7, 30.3, 32.1, 33.1, 36.8, 36.9, 37.7, 38.1, 50.0, 55.3, 70.0 ppm; all data are in close agreement with the literature;[24] IR (thin film) 3254, 2928, 2859, 1457, 1135, 1067, 976 cm^{-1}; HRMS (EI): *m*/*z* calcd for $C_{19}H_{37}NO$: 295.2870 $[M]^+$; found: 295.2871. A small portion was treated with an excess of HCl in MeOH (0.1 M) and then concentrated to give (−)-perhydrohistrionicotoxin (HCl salt) **2·HCl** $[\alpha]_D^{21} = -34.5$ (*c* 1.06, $CHCl_3$; lit. [4] −34.5).

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