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Investigation of conjugate addition/intramolecular nitrone dipolar cycloadditions and their use in the synthesis of dendrobatid alkaloid precursors †

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The sequential intramolecular conjugate addition of the oxime **13** followed by intramolecular dipolar cycloaddition of the intermediate nitrone **14** affords a mixture of the isoxazolidines **15**, **16** and **17**. The tricyclic 6,5,5-adduct **15** is believed to be the product of kinetic control and can be equilibrated with the epimeric tricyclic 6,5,5-isoxazolidine **17** through a β-elimination/conjugate addition process. Conditions have been developed for the two-step conversion of the ketone **12** under thermodynamic control into the racemic tricyclic 6,6,5-adduct **16** which is the core precursor of all the known histrionicotoxin alkaloids.

Introduction

The histrionicotoxins (*e.g.* HTX **1**, Fig. 1), a family of azospirocyclic-undecane containing alkaloids, were first isolated from the skins of the brightly coloured Colombian poison arrow frogs *Dendrobates histrionicus* and their structures were determined by Daly and coworkers.[2] They are important neurophysiological research tools which have aroused considerable interest as a result of their ability to act as highly potent and selective non-competitive inhibitors of the neuromuscular,[3,4] ganglionic and central neuronal nicotinic acetylcholine receptors.[5]

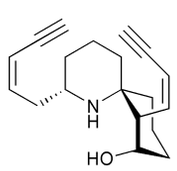

Fig. 1 (−)-Histrionicotoxin **1**.

Natural sources of the alkaloids can only provide a limited amount of histrionicotoxin derivatives (*ca.* 180 μg material per frog skin), and attempts to determine a plausible biosynthetic pathway have failed. Frogs raised in captivity do not appear to secrete the histrionicotoxins, indicating that they have developed systems which accumulate alkaloids from dietary sources such as ants and mites into the granular poison glands of their skin.[6–8] This, together with protection of the frogs under the CITES agreement (Convention on International Trade in Endangered Species), means that a rapid synthetic route is required.

Three total syntheses of histrionicotoxin have been reported.[1,9,10] Our route employed a sequential hydroxylamine-alkyne cyclisation/dipolar cycloaddition sequence that has produced a core tricyclic intermediate capable of conversion into all the known members of the HTX family. Many earlier attempts to synthesise the azaspirocycloundecane core of histrionicotoxin have employed an intramolecular 1,3-dipolar nitrone cycloaddition approach. Specifically the work of Tufariello,[11] Wehrli[12] and Grigg[13–18] and their coworkers had repeatedly demonstrated that the cycloaddition of substrates of general structure **2** (X ≠ H) invariably afforded the unwanted tricyclic 6,5,5-isoxazolidine **4** (Fig. 2). It had been proposed by Tufariello[11] and Wehrli[12] that the transition state **5** leading to the 6,5,5-adduct **7** was less crowded than the transition state **6** leading to the 6,6,5-adduct **8**. Wehrli was successful in converting the *unsubstituted* isoxazolidine **7** (R = H) into **8** (R = H), but no such experiments had been reported with analogues in which R = alkyl. Until our own work the formation of the substituted isoxazolidine (R ≠ H) by intramolecular nitrone cycloaddition had not proven possible (Fig. 3). Our solution to this problem was twofold. We formed the nitrone *in situ* by dipolar cycloreversion of styrene from **9** (Fig. 4) and used a cyano substituent in the dipolarophile **10**. The outcome was a highly regioselective formation of **11**.[1] This approach has allowed many members of the HTX family to be synthesised in good yield.[19]

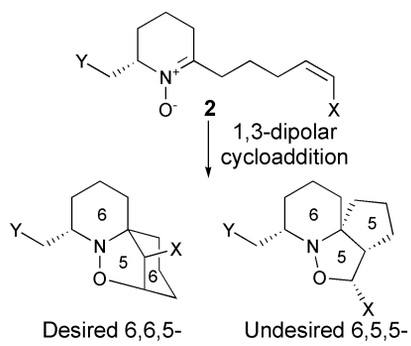

Fig. 2 Regiochemistry of dipolar cycloaddition of **2**.

Stockman[20] then elegantly combined the important cyano substituent on the dipolarophile with the extensive Grigg approach to *in situ* nitrone formation in a two-directional approach starting from the oxime **13** of the ketone **12**. Conjugate addition yielded the nitrone **14** followed by cycloaddition to produce racemic **16** in 47% overall yield.[20] Very recently more details of this work have been reported in a full paper.[21] The

† Nitrone dipolar cycloaddition routes to piperidines and indolizidines. Part 10. For part 9, see ref. 1.

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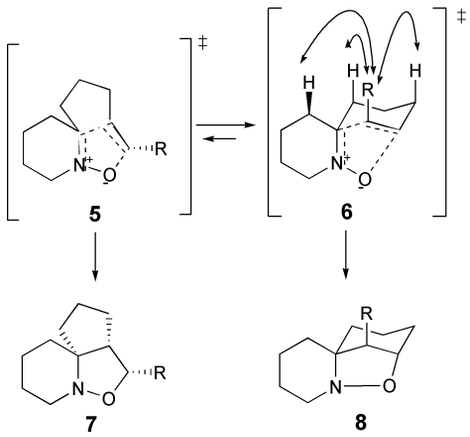


Fig. 3 Transition states **5** and **6**.

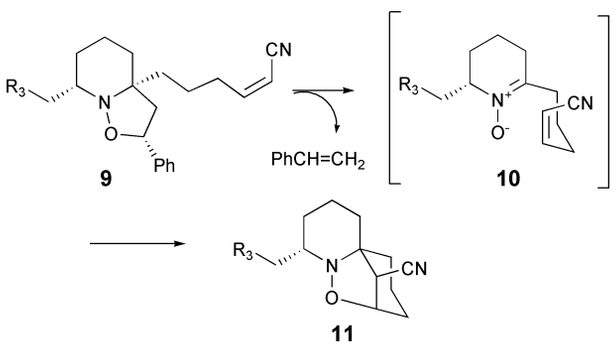


Fig. 4 Nitrone route to HTX core **11**.

isoxazolidine **15** was shown to be an intermediate in the conversion of the oxime **13** into the 'thermodynamically favored' isoxazolidine **16** (overall yield 51%). Independently we have investigated the formation of the tricyclic dinitrile **16** from **13** (Fig. 5). Our results confirm Stockman's basic findings. We now report that the interconversion of intermediates is a very subtle process and we have identified conditions for the selective formation of the 6,5,5- or 6,6,5-isoxazolidines in excellent yield.

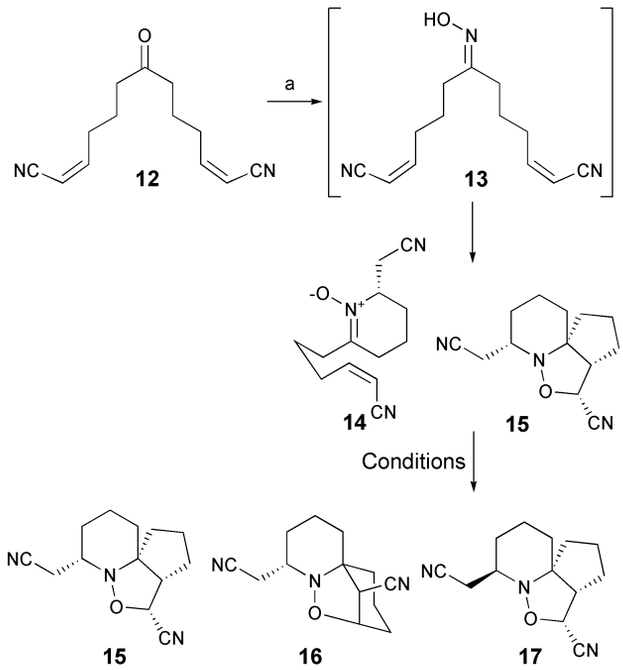
The Stockman approach offers a convenient opportunity to realise large quantities of synthetic intermediates for other members of the HTX family and to explore the subtle interplay between kinetic and thermodynamic control in intermediates such as **15** and **16**.

Results and discussion

The ketone **12** was prepared in 21% yield in five steps from 1,3-dithiane.[20] The *Z*,*Z*-unsaturated dinitrile was contaminated with approximately 8–12% of the *Z*,*E*-isomer owing to the incomplete stereoselectivity in the Yamamoto–Peterson reaction.[1] Treatment with hydroxylamine hydrochloride in methanol at room temperature gave a 1 : 1 mixture of the nitrone **14** and the tricyclic adduct **15** as determined by ^{1}H NMR analysis (Fig. 5). This demonstrates that the energy required for the cascade conjugate addition/intramolecular nitrone dipolar cycloaddition is relatively low and that cycloaddition can occur at room temperature.

Heating the 1 : 1 mixture of the tricyclic 6,5,5-isoxazolidine **15** and the nitrone **14** in both toluene and chlorobenzene gave a mixture of three tricyclic isoxazolidines **15**, **16** and **17**, together with small quantities of epimeric adducts believed to be derived from the (*Z*,*E*)-α,β-unsaturated nitrile (Fig. 5). Assignment of structures follows from X-ray crystal structures. The observation of the product **17**, the result of apparent intramolecular dipolar cycloaddition to the more hindered face of the nitrone was surprising and merited further investigation.

The results indicate that the ratio of products obtained varies according to concentration, solvent and temperature. Heating



Entry	Conditions	% **15** & epimers	%6,6,5 **16**	%6,5,5 **17**
1	Toluene, sealed tube, 160 °C, 2 h (7.1 mM)	14	25	23
2	Toluene, sealed tube, 160 °C, 2 h (9.3 mM)	24	17	35
3	Chlorobenzene, sealed tube, 180 °C, 2 h	10	27	13
4	Reflux toluene, 3 h	52 (**15**)	30	18
5	Reflux chlorobenzene, 3 h	33	18	34

Fig. 5 *Reagents and conditions*: (a) $NH_2OH.HCl$, NaOAc, MeOH, 25 °C.

the mixture of **14**/**15** in chlorobenzene at reflux (entry 5) gave a 1 : 2 : 2 ratio of the 6,6,5-tricycle **16** and the two 6,5,5-tricycles **15** and **17**, whilst refluxing in toluene (entry 4) afforded the 6,5,5-tricycle **15** as the major product. Heating at 160 °C in a sealed tube in toluene (entries 1, 2) gave either the 6,6,5-tricycle **16** or the 6,5,5-tricycle **17** as the major product depending on concentration. Heating at 180 °C in chlorobenzene in a sealed tube (entry 3) gave the required 6,6,5-tricycle **16** as the major product in a similar yield to that obtained from heating in a sealed tube in toluene. These results show a broad trend in which formation of the 6,5,5-tricycle **15** is favoured at lower temperatures, **17** at intermediate temperatures, and finally **16** at high temperatures.

The structures of these tricyclic adducts were confirmed by X-ray crystal structure analysis and 2-D NOE studies of the ^{1}H NMR spectra (Fig. 6).

After much experimentation optimum conditions were found for the conversion of the ketone **12** into the 6,5,5-adduct **15** in excellent yield (90%). The ketone **12** forms the oxime **13** and the nitrone **14** at room temperature. The nitrone undergoes a 1,3-dipolar cycloaddition to partially form the 6,5,5-adduct **15**. Heating this mixture at 50 °C pushes the equilibrium towards the 6,5,5-adduct **15**. The (*Z*,*E*)-isomer of the ketone is also converted into the 6,5,5-tricycle **15** thus maximising the yield, with the presumed C-6 cyano epimer of **15** being formed from the statistical presence of *ca.* 1% *E*,*E*-isomer of **12**.

The isomeric 6,5,5 tricyclic products **15** and **17** can be separated and individually converted by thermal equilibration in a

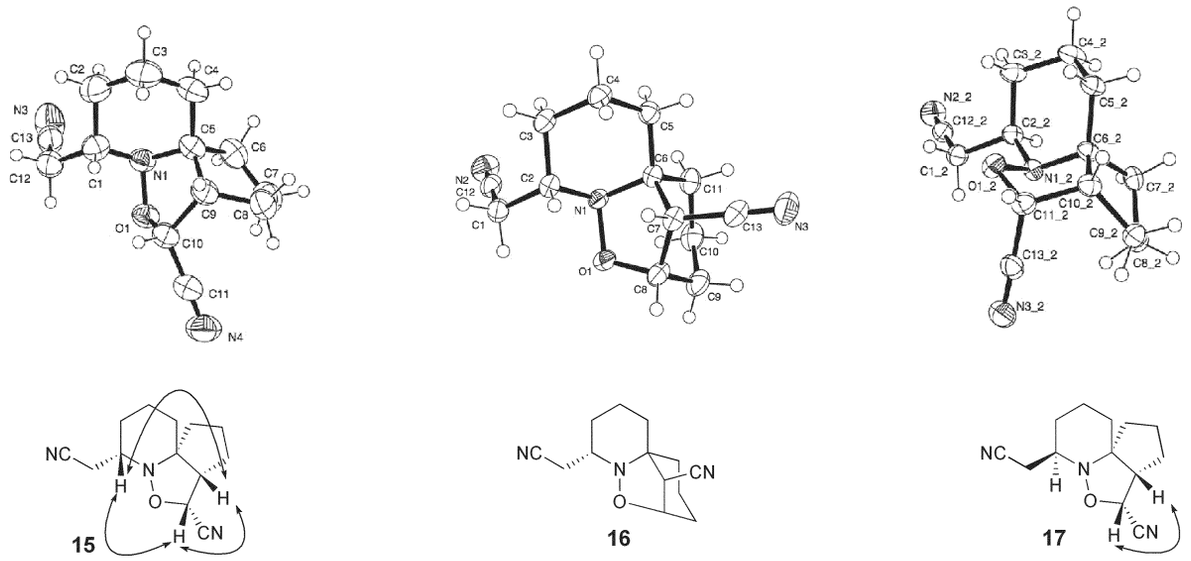


Fig. 6 ORTEP plots of X-ray structures and selected NOE data for the tricyclic adducts **15** (ref. 21), **16** (ref. 1) and **17**.

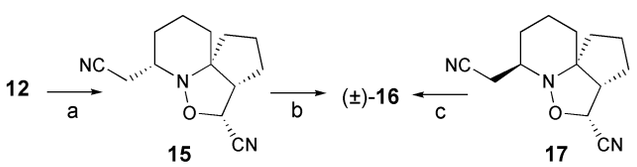


Fig. 7 *Reagents and conditions*: (a) i) $NH_2OH.HCl$, NaOAc, MeOH, ii) 50 °C, 90%; (b) chlorobenzene, sealed tube, 2 h, 180 °C, 83%; (c) chlorobenzene, sealed tube, 2 h, 180 °C, 88%.

sealed tube in chlorobenzene at 180 °C into the desired 6,6,5-adduct **16** in excellent yield (Fig. 7).

In order to follow the progress of the thermal equilibrations they were also conducted in a microwave oven where samples could easily be withdrawn at short intervals and monitored by LC/MS. The mixtures of nitrone **14** and tricyclic adduct **15** were subjected to five cycles of irradiation 600 s, 900 s, 2 × 1200 s and 1500 s in toluene and chlorobenzene (7.8 mM, max. power intake of sample 250 W). Heating in toluene at 110 °C and 160 °C, and chlorobenzene at 130 °C and 180 °C mimicked respectively the reactions carried out at reflux and in the sealed tube, which had illustrated that temperature was an important factor. Heating in toluene at 110 °C resulted in formation of the 6,5,5-adduct **15** as the major product with a small percentage conversion into the alternative 6,5,5-adduct **17** after 5400 s (Fig. 8). The evolution of the reaction in toluene at 160 °C is illustrated in Fig. 9 where the presence of **16** is evident after 2700 s and almost predominant after 3900 s.

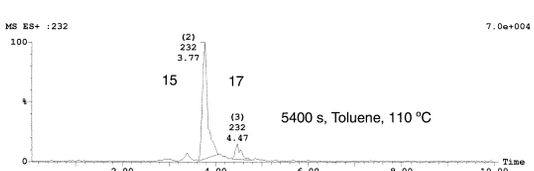


Fig. 8 LC/MS analysis of the thermal equilibration of **14** and **15** under microwave irradiation in toluene at 110 °C.

The results in chlorobenzene confirm that the 6,5,5-adduct **15** was converted through the epimeric 6,5,5-adduct **17** and then into the 6,6,5-adduct **16**. At 130 °C the two 6,5,5-adducts **15** and **17** were the major products after 5400 s (Fig. 10), while at 180 °C the 6,5,5-adduct **15** was converted solely into the required 6,6,5-adduct **16** after being cycled through the alternative 6,5,5-adduct **17** (Fig. 11).

These results demonstrate that the 6,5,5-adduct **15** that is formed at room temperature is the product of kinetic control, that the alternative 6,5,5-adduct **17** is a more stable product

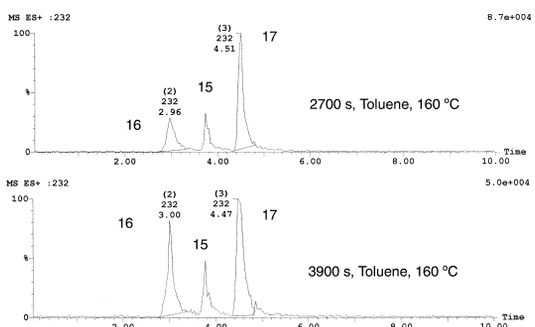


Fig. 9 LC/MS analysis of the thermal equilibration of **14** and **15** under microwave irradiation in toluene at 160 °C.

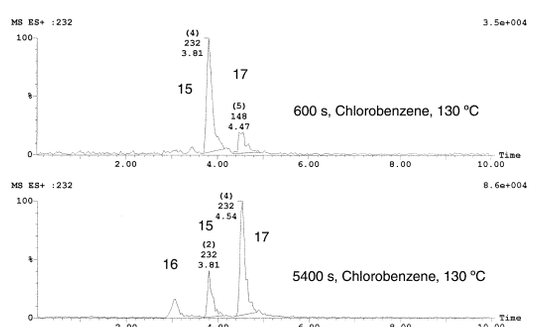


Fig. 10 LC/MS analysis of the thermal equilibration of **14** and **15** under microwave irradiation in chlorobenzene at 130 °C.

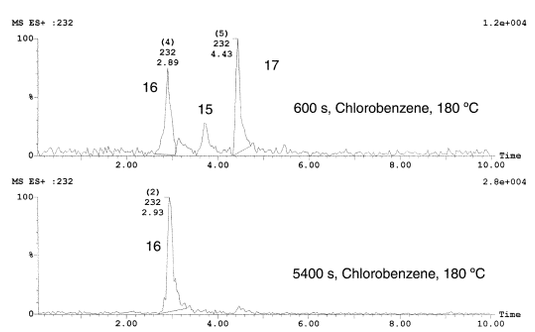


Fig. 11 LC/MS analysis of the thermal equilibration of **14** and **15** under microwave irradiation in chlorobenzene at 180 °C.

formed only by heating to >110 °C, and that the required 6,6,5-adduct **16** is only formed at high temperatures and is the product of thermodynamic control.

The geometries of **15**, **16** and **17** were fully optimized at the B3LYP[22] level using the standard 6–31G*[23] basis set. All quantum mechanics calculations were carried out with Jaguar.[24] Geometry optimisations were performed starting from the corresponding X-ray structures and the resulting calculated geometries were found to be very close to those obtained from X-ray diffraction. Structures **15** and **16** showed the same configuration on the sp^3 nitrogen, while **17** had the opposite configuration. Its energy increased by 2.2 kcal mol^{-1} when calculated with the same configuration as **15** and **16** (Fig. 12).

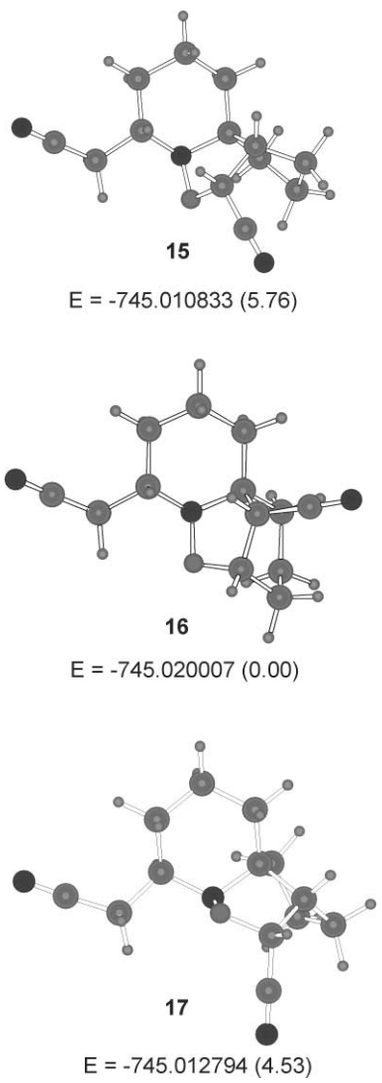


Fig. 12 B3LYP/6–31G* structures and energies of **15**,**16** and **17**. Total energies are given in hartrees and relative energies in kcal mol^{-1} (in parentheses).

The calculation of relative energies confirms that the desired 6,6,5-adduct **16** is the most stable, with the 6,5,5-adduct **15** being the least stable; the 6,5,5-adduct **17** was somewhere between the two, nearer to **15** than to **16**. From this it is reasonable to propose that the 6,6,5-adduct **16** is formed under thermodynamic control whereas **15** is the product of kinetic control. It is at first sight difficult to rationalise why the tricyclic adduct **17** should be favoured at all since it is formally derived by approach of the dipolarophile to the apparently more hindered face of the nitrone. However, it is possible to explain the equilibration of the 6,5,5-tricycles by a non-dipolar cycloaddition pathway.

We propose that at elevated temperatures the 6,5,5-tricycle **15** undergoes β-elimination followed by conjugate re-addition to form the epimer **17** (Fig. 13). The advantage of this explanation

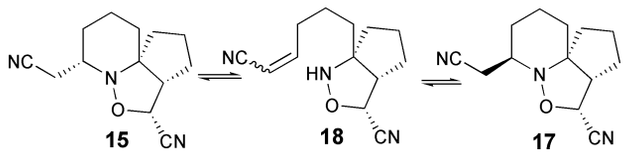


Fig. 13 Explanation for the equilibration of the 6,5,5-tricycles **15** and **17**.

is that the outcome is independent of the stereochemistry of the intermediate α,β-unsaturated nitrile **18** and allows for the efficient conversion of both (*Z,Z*) and (*Z,E*) isomers eventually into the tricyclic 6,6,5-adduct. This proposal also accounts for the interconversion of *both* **15** and **17** into the 6,6,5-tricycle **16**. Dipolar cycloreversion of either **15** or **17** affords a nitrone which under conditions of thermodynamic control will undergo a re-addition process in which the dipolarophile approaches the less-hindered face of the nitrone (*i.e.* opposite the pendant CH_2CN substituent) to give the more stable adduct (Fig. 14).

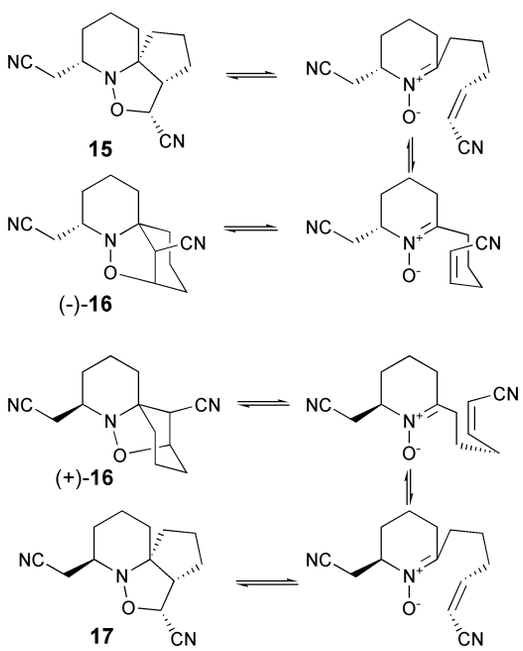


Fig. 14 Rationalisation of the equilibration of the tricyclic structures **15**, **17** and **16**.

Such an interconversion can only occur without noticeable stereochemical consequences if it is carried out on racemic substrates. Therefore a test of the hypothesis would be that equilibration of a single antipode of structure **16** should result in racemisation through formation of the enantiomer through the pathway depicted in Fig. 14. Resolution of the racemic dinitrile **16** on a semi-preparative chiral HPLC column (see Experimental section) afforded enantiomerically pure (+) or (−) **16** ($[a]_D^{24.5}$ +234; $[a]_D^{24.5}$ −220).[1] Subjection of the dextrorotary enantiomer (>98% ee) to the equilibration conditions (chlorobenzene, sealed tube, 185 °C, 16 h) and monitoring of the reaction mixture by analysis on a chiral analytical hplc column (see experimental section) showed the slow appearance of the other enantiomer (Fig. 15, (+)-**16** retention time 8.790 min, (−)-**16** retention time 10.257 min) thus confirming the hypothesis.

Other workers[25] have epimerised a stereocentre alpha to the nitrogen atom in isoxazolidines formed by intramolecular nitrone dipolar cycladditions by first reductively cleaving the N–O bond and then epimerising the centre through β-elimination. The example illustrated in Fig. 13 is to our knowledge the first observation of β-elimination – conjugate re-addition of an isoxazolidine.

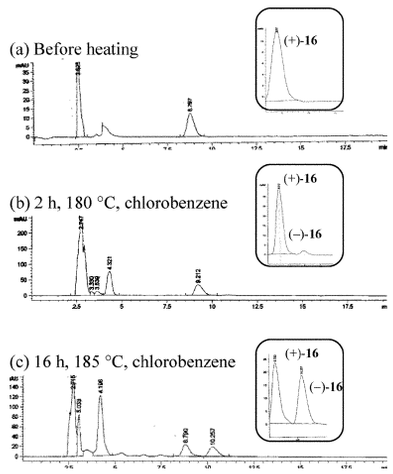


Fig. 15 Chiral HPLC analysis of the thermal racemisation of (+)-**16**.

Crystallographical data

X-Ray crystal data§ for compounds **15**, **16** and **17**. *Crystal data:* **15**: $C_{13}H_{17}N_3O$, M=231.30, monoclinic, space group $P2_1/n$ (no. 14), a = 12.610(3), b = 12.464(3), c = 16.763(3) Å, β = 107.01(3)°, U = 2519.5(10) Å^3, Z = 8, μ(Mo-Kα) = 0.080 mm^{-1}, 21461 reflections measured at 180(2) K using an Oxford Cryosystems Cryostream cooling apparatus, 5684 unique (R_{int} = 0.03); R_1 = 0.048, wR_2 = 0.044 [I>2σ(I)]; goodness-of-fit on F^2, S=1.175. The structure was solved and refined with CRYSTALS* (CCDB 230766).¶[26] **16**: $C_{13}H_{17}N_3O$, M=231.30, monoclinic, space group Cc (no. 9), a = 15.5820(9), b = 9.9509(6), c = 7.9189(5) Å, β = 97.128(3)°, U = 1218.4(1) Å^3, Z = 4, μ(Mo-Kα) = 0.083 mm^{-1}, 3327 reflections measured at 180(2) K using an Oxford Cryosystems Cryostream cooling apparatus, 1725 unique (R_{int} = 0.029); R_1 = 0.031, wR_2 = 0.072 [I>2σ(I)]; goodness-of-fit on F^2, S = 1.098. The structure was solved with SHELXS-97* and refined with SHELXL-97*[27] (CCDB 230717). **17**: $C_{13}H_{17}N_3O$, M = 231.30, triclinic, space group P-1 (no. 2), a = 5.8416(1), b = 6.9482(2), c = 31.712(2) Å, a = 86.981(1), β = 89.733(1), γ = 68.635(1)°, U = 1196.9(1) Å^3, Z = 4, μ(Mo-Kα) = 0.084 mm^{-1}, 7169 reflections measured at 180(2) K using an Oxford Cryosystems Cryostream cooling apparatus, 4054 unique (R_{int} = 0.052); R_1 = 0.060, wR_2 = 0.121 [I>2σ(I)]; goodness-of-fit on F^2, S = 1.145. The structure was solved with SHELXS-97* and refined with SHELXL-97*[27] (CCDB 230718).

Conclusion

A significantly improved yield (78%) of **16** the racemic tricyclic 6,6,5-core dinitrile common intermediate to all the known histrionicotoxins has been developed by the bidirectional Stockman approach from the ketone **12**. A combination of isolation of key intermediates, X-ray crystallography, molecular modelling and thermal equilibration under microwave irradiation have allowed a mechanistic rationalisation to be advanced for this complex process. Furthermore it offers insight into possible methods of exploiting these reactions for enantioselective synthesis using alternative dipolarophiles.

Experimental

Experimental details have been described previously.[1b] ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 (400 MHz, 100 MHz), Bruker DPX-500 (500 MHz, 125 MHz) or Bruker AVANCE-700 (700 MHz, 175 MHz) instruments. Infrared spectra were recorded on Perkin-Elmer Spectrum One ATR-FTIR spectrometer. HPLC studies were carried out on a Hewlett-Packard HP 1090 series HPLC machine. A normal phase silica column (Daicel Chiralpak^R AS amylose chromatography column, 20025, 0.46 cm ø × 25 cm) with silica guard tube (Daicel Chiralpak^R AS amylose chromatography column, 20022, 0.46 cm ø × 5 cm) was used for analysis. HPLC traces were recorded by eluting with 15% ethanol/hexane at a flow rate of 1.5 mL min^{-1} with an injection volume of 5 μL. Semi-preparative HPLC was carried out on a Varian Prostar HPLC system. A normal phase silica column (Daicel Chiralpak^R AS amylose chromatography column, 2 cm ø × 25 cm) was used for preparative separation. HPLC traces were recorded by eluting with 15% ethanol/isohexane at a flow rate of 5 mL min^{-1} with an injection volume of between 250–475 μL. Product was detected with a Varian ProStar UV-Vis detector, Model 320, set at 210 nm. Mass spectra, electron impact (EI) and chemical ionisation (CI), were recorded by the EPSRC National Mass Spectrometry service centre at the University of Wales, Swansea. Microwave studies were carried out using a Smith Synthesizer, with a maximum power intake of the sample of 250 W. LC/MS were carried out using a VG Platform with a Waters ZQ2000 mass spectrometer and a flow rate of 1 cm^3 min^{-1}. LC/MS column, 3 micron Supelco ABZ + 33 × 4.6 mm, run with acetonitrile + 0.1% aqueous formic acid. Flow injection starts at 1 cm^3 min^{-1} 20% acetonitrile/aqueous formic acid for 1 minute, followed by ramping to 90% acetonitrile/aqueous formic acid over 4 minutes.

7-Oxo-trideca-2(*Z*),11(*Z*)-diene dinitrile 12

The ketone **12** was obtained in 21% overall yield by the procedure described by Stockman:[20] colourless oil; R_f 0.19 (7 : 3 ether : petroleum ether); δ_H ($CDCl_3$, 500 MHz) 6.48 (2 H, dt, J 11 and 8, C*H*=CHCN), 5.37 (2 H, dt, J 11 and 1, CH=C*H*N), 2.49 (4 H, t, J 7, CH_2COCH_2), 2.45 (4 H, ddt, J 1, 8 and 8, CH_2-CH=CHCN), and 1.80 (4 H, quintet, J 7 and 7, CH_2CH$_2$CH=CHCN); data identical to literature values.[20,21] Key signal of (*E,Z*)-isomer; δ_H ($CDCl_3$, 500 MHz) 6.70 (1 H, dt, J 16 and 7, C*H*=CHCN); (*Z* : *E*) 8–10%.

(1*S*,5*S*,6*R*,9*S*) and (1*R*,5*R*,6*S*,9*R*)-9-cyanomethyl-6-cyano-8-aza-7-oxatricyclo[6.4.0.0^{1,5}]dodecane 15

(1*R*,5*S*,8*S*,12*R*) and (1*S*,6*R*,8*R*,12*S*)-5-cyanomethyl-12-cyano-6-aza-7-oxatricyclo[6,3,1,0^{1,6}]undecane 16

(1*R*,5*R*,6*S*,9*S*) and (1*S*,5*S*,6*R*,9*R*)-9-cyanomethyl-6-cyano-8-aza-7-oxatricyclo[6.4.0.0^{1,5}]dodecane 17

7-Oxo-trideca-2(*Z*),11(*Z*)-diene dinitrile **12** (121 mg, 0.560 mmol) was dissolved in methanol (47.0 cm^3) and added to hydroxylamine hydrochloride (52.0 mg, 0.750 mmol) and anhydrous sodium acetate (122 mg, 1.49 mmol). The mixture was stirred under nitrogen at room temperature for 24 h, whereupon TLC indicated that a significant proportion of nitrone **13** had been formed, the impurity being the 6,5,5 adduct. The reaction mixture was quenched with brine (50 cm^3) and extracted with CH_2Cl_2 (3 × 50 cm^3), water being added to dissolve all salts. The organic layers were combined, dried ($MgSO_4$/Na_2SO_4) and the solvent was removed *in vacuo* to give the crude product as a brown oil which was used crude.

Method 1. The crude nitrone **13** (28.0 mg, 0.120 mmol) was dissolved in CH_2Cl_2 and transferred to a thick walled glass tube, and the solvent was removed under a stream of nitrogen. Chlorobenzene (20 cm^3) was added and the reaction vessel was sealed with a Teflon screw cap. The reaction vessel was heated to 185 °C for 2 h before being allowed to cool to room temperature. The reaction mixture was diluted with toluene and the

§ CCDC reference numbers 230717, 230718 and 230766. See http://www.rsc.org/suppdata/ob/b4/b402307b/ for crystallographic data in.cif or other electronic format.

¶ The structure of a different polymorph of **15** (in space group $C2/c$) has been reported by Stockman.[21] These two polymorphs have slightly different melting points and identical solution ^{13}C NMR spectra. However, the signal at δ 2.55 in our ^{1}H NMR spectrum measured at 700 MHz is dd (J 17 and 8).

solvent was removed *in vacuo* to give the crude product as a brown solid which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to give the 6,6,5 adduct **16** (7.6 mg, 0.0329 mmol, 27%) as a colourless crystalline solid, the 6,5,5 adduct **17** (3.5 mg, 0.0151 mmol, 13%) as a colourless crystalline solid and a mixture of alternative epimers including the tricycle **15** (2.9 mg, 0.0125 mmol, 10%).

Method 2. The crude nitrone **13** (43.0 mg, 0.187 mmol) was dissolved in CH_2Cl_2 and transferred to a thick walled glass tube, and the solvent was removed under a stream of nitrogen. Degassed toluene (20 cm^3) was added and the reaction vessel was sealed with a Teflon screw cap. The reaction vessel was heated to 160 °C for 2 h before being allowed to cool to room temperature. The reaction mixture was diluted with toluene and the solvent was removed *in vacuo* to give the crude product as a brown oil which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to give the 6,6,5 adduct **16** (7.4 mg, 0.0320 mmol, 17%) as a colourless crystalline solid, the 6,5,5 adduct **17** (14.8 mg, 0.0640 mmol, 35%) as a colourless crystalline solid and a mixture of alternative epimers including tricycle **15** (10.7 mg, 0.0463 mmol, 24%).

Method 3. The crude nitrone **13** (43.0 mg, 0.187 mmol) was dissolved in chlorobenzene (25 cm^3) and was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo* to give the crude product as a brown oil which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to yield the 6,6,5 adduct **16** (7.8 mg, 0.0340 mmol, 18%) as a colourless crystalline solid, the 6,5,5 adduct **17** (14.9 mg, 0.0640 mmol, 34%) as a colourless crystalline solid, and a mixture of alternative epimers including the 6,5,5 adduct **15** (14.3 mg, 0.0620 mmol, 33%).

Method 4. The crude nitrone **13** (43.0 mg, 0.187 mmol) was dissolved in degassed toluene (25 cm^3) and heated under reflux for 3 h. The reaction mixture was cooled to room temperature and the solvent was removed *in vacuo* to give the crude product as a brown oil which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to give the 6,6,5 adduct **16** (13.5 mg, 0.0584 mmol, 30%) as a colourless solid which was recrystallised from hexane : ethyl acetate, the 6,5,5 adduct **17** (8.3 mg, 0.0359 mmol, 18%) as a colourless solid which was recrystallised from hexane : ethyl acetate, and the 6,5,5 adduct **15** as colourless plates which were recrystallised from hexane : ether (24.8 mg, 0.107 mmol, 55%).

Data for **16**: R_f 0.19 (2 : 1 isohexane : ethyl acetate); mp (hexane : ethyl acetate) 125–127 °C, δ_H(400 MHz, $CDCl_3$) 4.73 (1 H, ddd, *J* 3, 3 and 6, C*H*O), 3.36 (1 H, dd, *J* 2 and 6, C*H*CN), 2.76 (1 H, dd, *J* 3 and 17, C*H*HCHN), 2.77–2.68 (1 H, m, C*H*CN), 2.55 (1 H, dd, *J* 8 and 17, CH*H*CHN), 2.25–2.20 (1 H, m), 1.96–1.82 (3 H, m), 1.79–1.54 (6 H, m) and 1.47–1.31 (2 H, m); data identical to literature values.[10]

17 R_f 0.26 (2 : 1 isohexane : ethyl acetate); mp 105–107 °C (hexane : ethyl acetate); ν_{max} (thin film)/cm^{-1} 2942s (C-H), 2864m (C-H), 2251w (C≡N), 1447m, 1361w, 1339w, 1210w, 1134w, 1036s and 1017s; Found: C, 67.5; H 7.4; N, 18.0; $C_{13}H_{17}N_3O$ requires C, 67.5; H, 7.4; N, 18.2%; δ_H(500 MHz, $CDCl_3$) 4.92 (1 H, br d, *J* 8, C*H*O), 3.28 (1 H, br s, C*H*N), 2.81–2.75 (2 H, m, C*H*HCN, C*H*CH(O)CN), 2.64 (1 H, dd, *J* 8 and 17, C*H*HCN), 2.10 (1 H, br s), 1.98–2.03 (1 H, m), 1.62–1.92 (6 H, m) and 1.37–1.61 (4 H, m); δ_C(125 MHz, $CDCl_3$) 117.9 (s), 117.1 (s), 74.4 (s), 69.7 (d). 58.6 (d), 55.4 (d), 33.8 (t), 30.3 (t), 28.9 (t), 27.0 (t), 24.0 (t), 23.0 (t) and 21.8 (t); *m*/*z* (ES^+) 249.1709 ($[M + NH_4]^+ \cdot C_{13}H_{21}ON_4$ requires *M* 249.1710); *m*/*z* (CI) 250 (14), 249 ($[M + NH_4]^+$, 100), and 232 (8).

15; R_f 0.14 (2 : 1 isohexane : ethyl acetate); mp 76–77 °C (hexane : ether); ν_{max} (thin film)/cm^{-1} 2944s (C-H), 2879s (C-H), 2249s (C≡N), 1459w, 1447m, 1421w, 1371w, 1350w, 1169w and 1072s; Found: C, 67.6; H, 7.6; N, 17.9; $C_{13}H_{17}N_3O$ requires C, 67.5; H, 7.4; N, 18.2%; δ_H(700 MHz, $CDCl_3$) 4.99 (1 H, d, *J* 10, C*H*O), 2.85 (1 H, dd, *J* 8 and 8, C*H*CHCN), 2.79 (1 H, dd, *J* 3 and 17, C*H*HCN), 2.70 (1 H, tq, *J* 3 and 8, C*H*N), 2.55 (1 H, dd, *J* 8 and 17, CH*H*CN), 2.21–2.17 (1 H, m, H-4), 2.09–1.87 (5 H, m), 1.82–1.71 (3 H, m), 1.67 (1 H, ddd, *J* 13, 8 and 8, H-2), 1.49 (1 H, dq, *J* 3 and 12, H-10), and 1.39 (1 H, tq, *J* 4 and 12, H-11); δ_C(175 MHz, $CDCl_3$) 117.6 (s), 115.8 (s), 78.3 (s), 68.7 (d), 58.1 (d), 50.7 (d), 40.2 (t), 32.8 (t), 28.8 (t), 28.7 (t), 23.4 (t), 22.9 (t) and 20.0 (t); *m*/*z* (ES^+) 232.1443 ($[M + H]^+ \cdot C_{13}H_{18}N_3O$ requires *M*, 232.1444); *m*/*z* (CI) 250 (25), 249 ($[M + NH_4]^+$, 100), 232 ($[M + H]^+$, 82), 216 (86), 207 (39), 205 (35), 191 (30) and 166 (92).

(1*S*, 5*S*, 6*R*, 9*S*) and (1*R*, 5*R*, 6*S*, 9*R*)-9-cyanomethyl-6-cyano-8-aza-7-oxatricyclo[6.4.0.0^{1,5}]dodecane 15

To the ketone **12** (243 mg, 1.12 mmol) dissolved in methanol (80 cm^3) was added sodium acetate (250 mg, 3.05 mmol) and hydroxylamine hydrochloride (86.0 mg, 1.238 mmol, 1.1 mol eq.). The mixture was stirred under nitrogen at room temperature for 21 h before being heated to 50 °C and stirred for 7 h. The reaction was quenched with ammonium chloride (30 cm^3) and diluted with CH_2Cl_2 (30 cm^3), brine was added in order to separate the aqueous and organic layers. The mixture was extracted with further CH_2Cl_2 (3 × 30 cm^3) and the organic layers combined, dried ($MgSO_4$) and concentrated *in vacuo* to yield the crude product which was purified by flash column chromatography (2 : 1 isohexane : ethyl acetate) to yield the product **15** as a colourless oil which could be crystallised from hexane : ether (235 mg, 1.02 mmol, 90%).

(1*R*,5*S*,8*S*,12*R*) and (1*S*,6*R*,8*R*,12*S*)-5-cyanomethyl-12-cyano-6-aza-7-oxatricyclo[6,3,1,0^{1,6}]undecane 16

Method 1. The 6,5,5 adduct **17** (26.6 mg, 0.115 mmol) was dissolved in CH_2Cl_2, transferred to a thick walled glass tube, and the solvent was removed under a stream of nitrogen. Degassed chlorobenzene (20 cm^3) was added and the reaction mixture was freeze–thaw degassed (three cycles) before being sealed with a Teflon screw cap. The reaction vessel was heated to 185 °C for 2 h 10 min before being left to cool to room temperature. The reaction mixture was diluted with toluene and the solvent was removed *in vacuo* to give the crude product as a brown oil which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to give the 6,6,5 adduct **16** (22 mg, 0.0952 mmol, 83%) as a colourless crystalline solid.

Method 2. The 6,5,5 adduct **15** (37.6 mg, 0.163 mmol) was dissolved in CH_2Cl_2, transferred to a thick walled glass tube, and the solvent was removed under a stream of nitrogen. Chlorobenzene (20 cm^3) was added and the reaction mixture was freeze–thaw degassed (three cycles) before being sealed with a Teflon screw cap. The reaction vessel was heated to 180 °C for 2 h before leaving to cool to room temperature. The reaction mixture was diluted with toluene and the solvent was removed *in vacuo* to give the crude product as a brown oil which was purified by flash column chromatography (2 : 1 hexane : ethyl acetate) to yield the 6,6,5 adduct **16** (33 mg, 0.143 mmol, 88%) as a colourless crystalline solid.

Microwave reactions

(1*S*,5*S*,6*R*,9*S*) and (1*R*,5*R*,6*S*,9*R*)-9-cyanomethyl-6-cyano-8-aza-7-oxatricyclo[6.4.0.0^{1,5}]dodecane 15

(1*R*,5*S*,8*S*,12*R*) and (1*S*,6*R*,8*R*,12*S*)5-cyanomethyl-12-cyano-6-aza-7-oxatricyclo[6,3,1,0^{1,6}]undecane 16

(1*R*,5*R*,6*S*,9*S*) and (1*S*,5*S*,6*R*,9*R*)-9-cyanomethyl-6-cyano-8-aza-7-oxatricyclo[6.4.0.0^{1,5}]dodecane 17

The crude nitrone **13** (5 mg, 0.0216 mmol) was dissolved in toluene (2.76 cm^3) and the microwave tube was sealed. The

mixture was then subjected to irradiation at 160 °C for the following time sequences: 600 s, 900 s, 2 × 1200 s, 1500 s. After each irradiation the mixture was analysed by LC/MS which indicated that tricycle **15** was first formed followed by tricycle **17** and finally the required tricycle **16**, confirmed by ^{1}H NMR analysis. ^{1}H NMR analysis indicated the ratio of tricycles as: 1 : 0.58 : 0.38, **16** : **15** : **17**, + 7% other uncharacterised 6,5,5-epimer. The key peaks integrated to determine the ratio of tricycles were **16** 4.73 (1 H, ddd, *J* 3, 3 and 6, C*H*O); **17** 4.92 (1 H, br d, *J* 8, C*H*O) and **15** 4.99 (1 H, d, *J* 10, C*H*O).

The crude nitrone **13** (5 mg, 0.0216 mmol) was dissolved in toluene (2.76 cm^3) and the microwave tube was sealed. The mixture was then subjected to irradiation at 110 °C for the following time sequences: 600 s, 900 s, 2 × 1200 s, 1500 s. After each irradiation the mixture was analysed by LC/MS which indicated that at this temperature tricycle **15** was formed with a minor amount of the alternative 6,5,5-isomer **17**, confirmed by ^{1}H NMR analysis. ^{1}H NMR analysis indicated the ratio of tricycles as: 1 : 0.07, **15** : **17**. The key peaks integrated to determine the ratio of tricycles were **16** 4.73 (1 H, ddd, *J* 3, 3 and 6, C*H*O); **17** 4.92 (1 H, br d, *J* 8, C*H*O) and **15** 4.99 (1 H, d, *J* 10, C*H*O).

The crude nitrone **13** (5 mg, 0.0216 mmol) was dissolved in chlorobenzene (2.76 cm^3) and the microwave tube was sealed. The mixture was then subjected to irradiation at 180 °C for the following time sequences: 600 s, 900 s, 2 × 1200 s, 1500 s. After each irradiation the mixture was analysed by LC/MS which indicated that tricycle **15** was first to be formed followed by tricycle **17** and finally the required tricycle **16**, confirmed by ^{1}H NMR analysis. ^{1}H NMR analysis indicated the ratio of tricycles as: 1 : 0.08, **16** : 6,5,5-epimer. The key peaks integrated to determine the ratio of tricycles were **16** 4.73 (1 H, ddd, *J* 3, 3 and 6, C*H*O); **17** 4.92 (1 H, br d, *J* 8, C*H*O) and **15** 4.99 (1 H, d, *J* 10, C*H*O).

The crude nitrone **13** (5 mg, 0.0216 mmol) was dissolved in chlorobenzene (2.76 cm^3) and the microwave tube was sealed. The mixture was then subjected to irradiation at 130 °C for the following time sequences: 600 s, 900 s, 2 × 1200 s, 1500 s. After each irradiation the mixture was analysed by LC/MS which indicated that at this temperature tricycle **15** was formed followed by the alternative 6,5,5-isomer **17** and finally the desired 6,6,5-tricycle **16**, confirmed by ^{1}H NMR analysis. ^{1}H NMR analysis indicated the ratio of tricycles as: 0.40 : 1 : 0.96, **16** : **15** : **17**, + 5% epimer. The key peaks integrated to determine the ratio of tricycles were **16** 4.73 (1 H, ddd, *J* 3, 3 and 6, C*H*O); **17** 4.92 (1 H, br d, *J* 8, C*H*O) and **15** 4.99 (1 H, d, *J* 10, C*H*O).

Racemisation studies

(1*R*,5*S*,8*S*,12*R*) and (1*S*,6*R*,8*R*,12*S*)5-cyanomethyl-12-cyano-6-aza-7-oxatricyclo[6,3,1,0^{1,6}]undecane 16

Method 1. The enantiomerically pure dextrorotary 6,6,5-adduct (+)-**16** was obtained by semi-preparative chiral HPLC on a Daicel Chiralpak^{R} AS amylose chromatography column, 2 cm ø × 25 cm, [*a*]+234.[10] The 6,6,5-adduct (+)-**16** (2.2 mg, 0.0095 mmol) was dissolved in chlorobenzene (1.6 cm^3) and the mixture was freeze–thaw degassed under argon (3 cycles) before being sealed with a Teflon screw cap. The reaction vessel was heated to 180 °C for 2 h 10 min before being left to cool to room temperature. The reaction mixture was washed out of the flask with toluene, and the solvent removed *in vacuo* to yield the product as a straw coloured oil which was purified by flash column chromatography (2 : 1 petroleum ether : ethyl acetate). The solvent was removed *in vacuo* to yield the crude product as a pale straw coloured oil (2 mg, 0.0087 mmol, 91%). The product was then dissolved in ethanol and subjected to HPLC analysis on an analytical Daicel Chiralpak^{R} AS amylose chromatography column, 20022, 0.46 cm ø × 5 cm as described previously. (+)-**16** retention time 9.212 min.

Method 2. The enantiomerically pure dextrorotatory 6,6,5-adduct (+)-**16** (2.0 mg, 0.0087 mmol) was dissolved in chlorobenzene (1.6 cm^3) and the mixture freeze–thaw degassed under nitrogen (3 cycles) before being sealed with a Teflon screw cap. The reaction vessel was heated to 185 °C for 16 h before being left to cool to room temperature. The mixture was diluted with toluene, and the solvent was removed *in vacuo* to yield the crude product as a straw coloured oil which was purified by flash column chromatography (2 : 1 petroleum ether : ethyl acetate). The solvent was removed *in vacuo* to yield the crude product as a pale straw coloured oil which was dissolved in ethanol and subjected to HPLC analysis as described previously. (+)-**16** retention time 8.790 min, (−)-**16** retention time 10.257 min.

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