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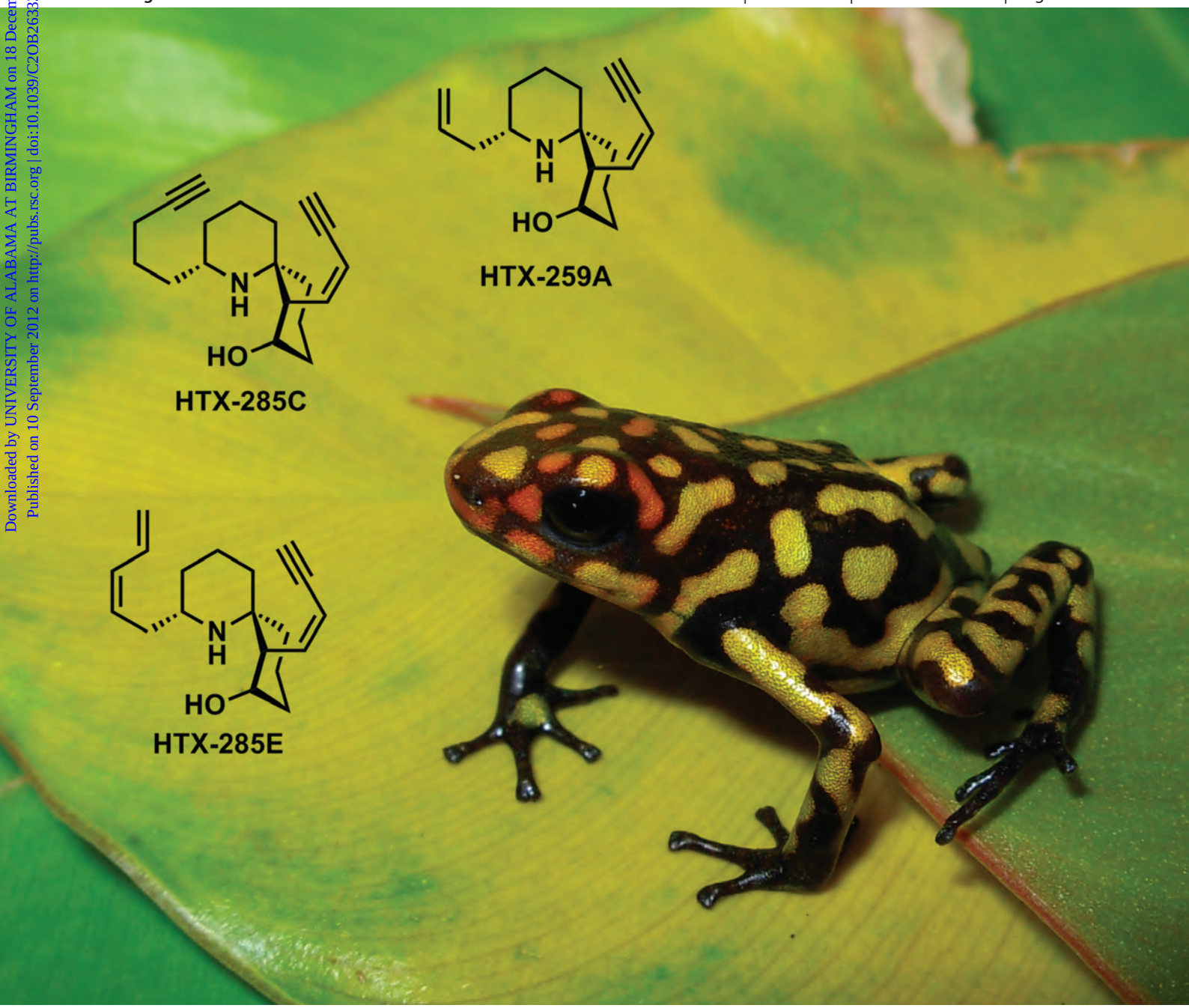

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PAPER

Intramolecular nitrone dipolar cycloadditions: control of regioselectivity and synthesis of naturally-occurring spirocyclic alkaloids†

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The intramolecular nitrone dipolar cycloaddition of *in situ*-generated nitrones such as compound **26** has been used for the synthesis of cyclic isoxazolidines **27** and **29**. The regioselectivity of the intramolecular cycloaddition depends on the nature of the terminal substituent on the dipolarophile. The influence of the substituent on the regioselectivity of the cycloaddition has been examined using several model systems and two methods of nitrone formation. These studies demonstrated that the cyano-substituent plays a special role in favouring the formation of the 6,6,5-ring fused adduct **27** under thermodynamically controlled conditions. The utility of the cyclo-adduct **57** (see Scheme 12) as a precursor for the naturally occurring histrionicotoxins is illustrated by the synthesis of three "unsymmetrical" (*i.e.* with each side chain bearing different functional groups) members of the histrionicotoxin family HTX-259A, HTX-285C and HTX-285E (**2**, **3** and **4** respectively).

Introduction

The histrionicotoxins are a family of alkaloids originating from skin extracts of the neotropical poison arrow frog *Dendrobates histrionicus*. The parent alkaloid (−)-histrionicotoxin (HTX-283A) **1** was first isolated by Daly and co-workers in 1971,[1] and since then a further 15 alkaloids of the same family have been isolated.[2,3] The various members of the family all share an azaspirocyclo-undecane core, and differ only in the length and degree of unsaturation present in the two side chains (Fig. 1). These alkaloids have attracted significant attention from the synthetic community.[4–12]

Over the years, there have been several contributions to this body of literature from our group,[7–9,13–16] including the only syntheses of the histrionicotoxins HTX-259A, HTX-285C and HTX-285E (**2**, **3** and **4** respectively), which bear differing side-chains.[17] Herein, we provide further detail of the synthetic work and describe the results of some investigations into the intriguing regioselectivity of dipolar cycloaddition observed during the key cascade hydroxylamine-alkyne cyclisation/nitrone cycloaddition to form the azaspirocyclic core.

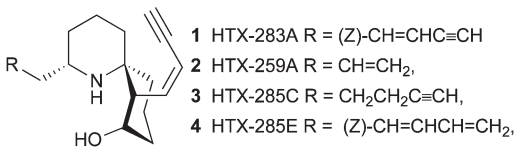

Fig. 1 Selected members of the histrionicotoxin family.

This intramolecular nitrone dipolar cycloaddition approach to the histrionicotoxins has attracted much interest.[4] The key to such a synthesis lies in the ability to control the regiochemistry of the cycloaddition to afford the desired 6,6,5-adduct **6**. Incorrect orientation of the intramolecular dipolarophile yields the undesired 6,5,5-adduct **7** (Fig. 2).[14]

Based on the results of Tufariello[18] and Gössinger,[19] who proposed that steric effects raise the energy of the transition state **5a** (Fig. 2), we had initially selected an ethynyl substituent on the alkene (**8**) to minimize these steric effects, but even this was apparently too bulky (or showed unfavourable HOMO–LUMO interactions) to allow the kinetic preference for the formation of the required adduct and the only observable product was the unwanted 6,5,5-isomer **10** (Scheme 1).[14]

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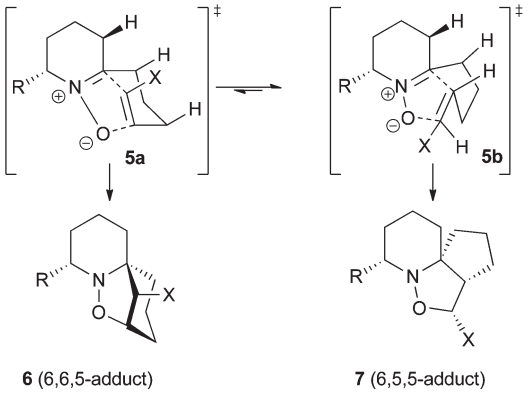
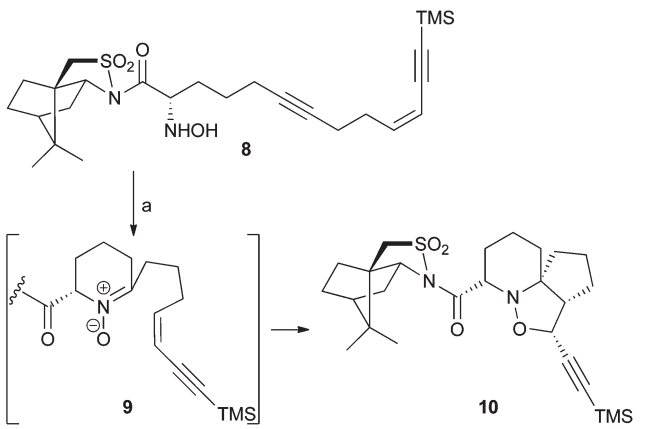


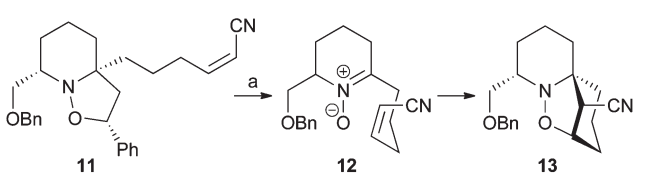
Fig. 2 Alternative transition states **5a** and **5b** leading to two different regioisomeric isoxazolidines **6** and **7** for the intramolecular dipolar cycloaddition of the *N*-alkenyl nitrone **5**.



Scheme 1 Nitrone cyclisation employing an ethynyl-substituted dipolarophile. *Reagents and conditions*: (a) PhMe, 110 °C, 83%.

When this approach proved unsuccessful, we selected a cyano group as a potentially even less sterically demanding substituent. A further benefit of the cyano substituent was the anticipation that it could lower the activation energy for the formation of the required 6,6,5-adduct by improving the frontier orbital interactions between the HOMO (dipole) and LUMO (dipolarophile).[13] This issue has recently been examined from a computational perspective.[20]

By generating the reactive nitrone functionality **12** *in situ* by the cycloreversion of the styrene adduct **11** in the presence of a pendant cyano-substituted dipolarophile, we found that it was possible to synthesise the required 6,6,5-adduct **13** in excellent yield (Scheme 2).



Scheme 2 Nitrone cyclisation employing cyano dipolarophile. *Reagents and conditions*: (a) PhMe, sealed tube, 190 °C, 80%.

The observed formation of the required 6,6,5-adduct **13** (Scheme 2) compared with the formation of the undesired 6,5,5-adduct **10** (Scheme 1) was of great interest to us, and we sought to understand whether this was owing to the use of the dipolar cycloreversion to generate the nitrone *in situ* or the cyano-substituent in the dipolarophile or a combination of both effects.

In this paper, we describe experiments to identify the causes of this surprising regiocontrol, which complement the recent reports of Stockman[21–23] and ourselves,[13] and show how this regiocontrol can be used to synthesise various natural histrionicotoxins.

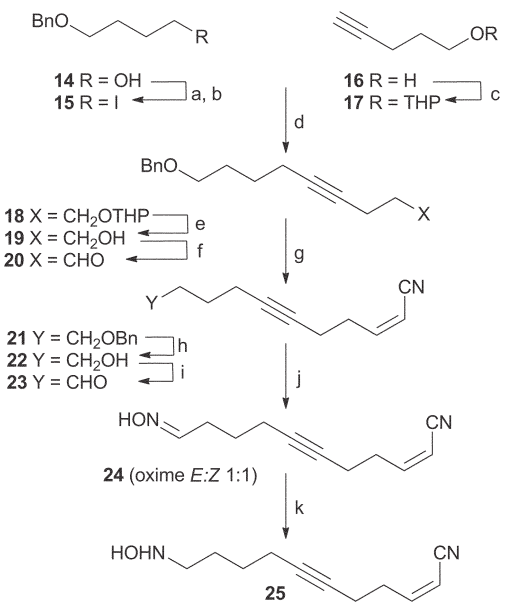
Results and discussion

Investigations into the regiochemical outcome of the intramolecular nitrone dipolar cycloaddition reaction

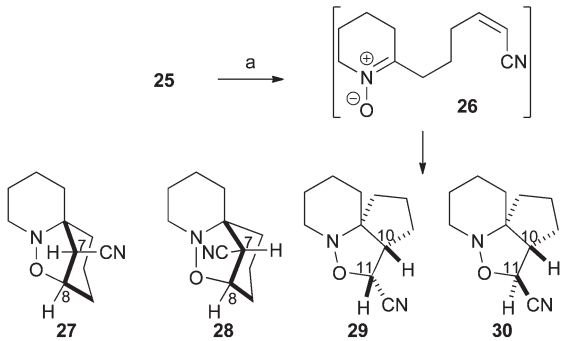
We set about examining the outcome of the nitrone dipolar cycloaddition reaction by examining a variety of model systems. These systems explored different methods for the formation of the nitrone, and compared the effects of incorporating a cyano-group with alternative substituents in the dipolarophile.

Model studies: formation of the nitrone by direct hydroxylamine-alkyne cyclisation

We initially examined formation of the nitrone *in situ* by employing a tandem hydroxylamine-alkyne cyclisation/dipolar nitrone



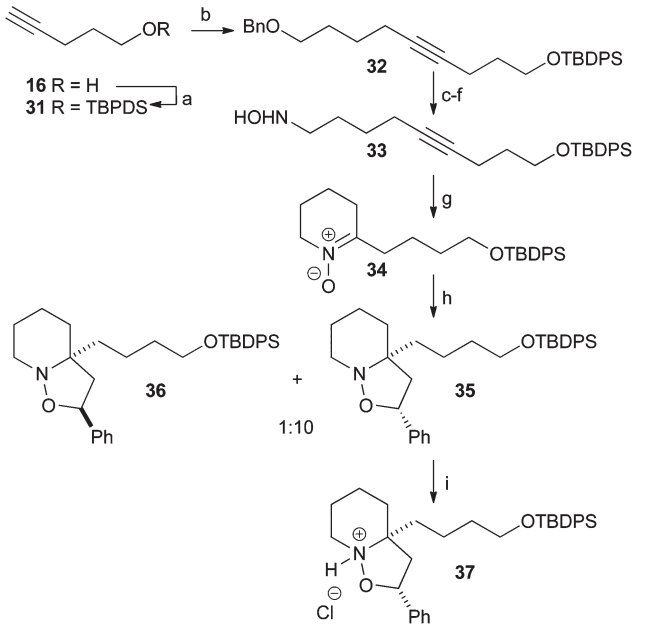
Scheme 3 Synthesis of the hydroxylamine **25**. *Reagents and conditions*: (a) TsCl, Et_3N, DMAP (cat.), CH_2Cl_2, 0 °C → 25 °C, 86%; (b) NaI, $Me_2C{=}O$, 91%; (c) 3,4-dihydro-2*H*-pyran, Amberlyst-15®, CH_2Cl_2, 96%; (d) (i) **17**, *n*-BuLi, THF, −10 °C; (ii) DMPU; (iii) **15**, THF, −10 °C → 25 °C, 88%; (e) Amberlyst-15®, MeOH, 97%; (f) (i) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C; (ii) Et_3N, −78 °C → 25 °C, 90%; (g) (i) $TMSCH_2CN$, *n*-BuLi, THF, −78 °C; (ii) $B(O^iPr)_3$; (iii) **20**, THF, −78 °C, 64%, (*Z*/*E* 6.8 : 1); (h) $BCl_3{\cdot}SMe_2$, CH_2Cl_2, 0 °C → 25 °C, 76%; (i) TPAP (cat.), NMO, activated 4 Å sieves, CH_2Cl_2, 0 °C → 25 °C, 94%; (j) $NH_2OH{\cdot}HCl$, NaOAc, EtOH/H_2O, 94%; (k) $NaBH_3CN$, HCl/MeOH, MeOH, methyl orange, pH 4, −15 °C.



Scheme 4 Tandem hydroxylamine–alkyne cyclisation/dipolar nitrone cycloaddition using **25**. *Reagents and condition*: (a) PhMe, 80 °C, 11% (1.25 : 1.25 : 2 : 1, **27** : **28** : **29** : **30**) (2 steps).

Table 1 Characteristic ^{1}H NMR spectroscopic signals for **27–30**

Compound	δ_H *C*7-H/*C*10-H	δ_H *C*8-H/*C*11-H
27	3.46, dd, *J* 2.3, 6.0	4.74, ddd, *J* 2.6, 3.1, 6.2
28	2.84, s	4.83, d, *J* 4.7
29	2.73, td, *J* 9.0, 3.0	4.92, d, *J* 9.0
30	2.40–2.60, m	4.24, d, *J* 5.3



Scheme 5 Protection of the cyclic nitrone as an isoxazolidine (styrene adduct) **35**. *Reagents and conditions*: (a) TBDPSCl, imidazole, CH_2Cl_2, 0 °C, 90%; (b) (i) **31**, *n*-BuLi, THF, −78 °C; (ii) **15**, 50 °C, 36 h, 88%; (c) $BCl_3{\cdot}SMe_2$, CH_2Cl_2, 16 h, 0 °C, 72%; (d) oxalyl chloride, DMSO, CH_2Cl_2, NEt_3, 89%; (e) $NH_2OH{\cdot}HCl$, sodium acetate, THF, 94%; (f) $NaCNBH_3$, MeOH, pH 3–4, 84%; (g) PhMe, 80 °C, 4.5 h; (h) styrene, quinol (2–3 mg), 85 °C, 16 h, 80% (2 steps); (i) HCl, MeOH (67%).

cycloaddition[24,25] in the presence of a pendant α,β-unsaturated nitrile which serves as the intramolecular dipolarophile. This system is analogous to our ethynyl substituted approach to the histrionicotoxins (Scheme 1). In order to test our hypotheses regarding the directing effects of the nitrile substituent, studies of model dipolar cycloadditions were performed on the *N*-alkenyl nitrone formed by hydroxylamine-alkyne cyclisation of the hydroxylamine **25**.

The hydroxylamine **25** was synthesised from commercially available starting materials **14** and **16** in 10 steps (Scheme 3). 4-Benzyloxybutan-1-ol (**14**) was tosylated, followed by a Finkelstein reaction[26] to furnish iodide **15** in high yield. The lithio-derivative of 4-pentyn-1-ol THP ether **17** was alkylated with **15** to afford the coupled product **18** in adequate yield. Removal of the THP group of **18** by treatment with Amberlyst-15® in methanol and subsequent Swern oxidation[27] of the resulting alcohol **19** gave the aldehyde **20**. The α,β-unsaturated nitrile **21** was then synthesised using Yamamoto's modification of the Petersen reaction.[28,29] The use of triisopropyl borate as a chelating agent resulted in a 6.8 : 1 ratio of the *Z*/*E* isomers (as determined by ^{1}H NMR spectroscopy) which were, unfortunately, inseparable by flash column chromatography. Debenzylation of **21** with boron trichloride–dimethylsulfide complex[30] occurred selectively in the presence of both alkene and alkyne functionalities to give the alcohol **22** which was oxidised to the aldehyde **23** with TPAP[31] followed by conversion into the oxime **24** in excellent yield. Reduction to the hydroxylamine **25** was accomplished under acidic conditions (pH 3–4) with sodium cyanoborohydride.[32]

Heating the hydroxylamine **25** (*Z*/*E*, 6.8 : 1) in toluene at 80 °C gave a complex mixture of products. After careful flash column chromatography, it was possible to isolate a pure sample of **29** as the major product albeit in very low yield (4%). This sample was characterised by ^{1}H NMR spectroscopy only. The observed signals matched those of a sample of **29** that we later synthesised *via* a different method and fully characterised (see Scheme 7). In addition to this material, a pure sample of another compound (2%) was isolated and characterised by ^{1}H NMR spectroscopy. By analogy with Horsley's work[13] and comparison with the data corresponding to similar compounds, we propose that this compound is the diastereomer **30**. The remaining mixed fractions appeared to contain a mixture of **27** and another unidentified adduct (5%). The signals were consistent with those of a pure sample of **27** which we isolated later (see Scheme 7). By analogy with Horsley's work,[13] we propose that the other unidentified adduct is diastereomer **28** (Scheme 4).

The low yield of the products was attributed to polymerisation *via* intermolecular conjugate addition of the hydroxylamine to the α,β-unsaturated nitrile functionality which was consistent with observation by TLC analysis of a highly polar degradation product. We propose that the diastereomeric adducts 6,6,5-(**28**) and 6,5,5-(**30**) were formed by the cycloaddition of the minor (*E*)-isomer of the α,β-unsaturated nitrile **25**. Thus the dipolar cycloaddition reaction appears to be stereospecific (concerted) as was also observed for cyanomethyl-substituted analogue of the nitrone **26**.[21–23]

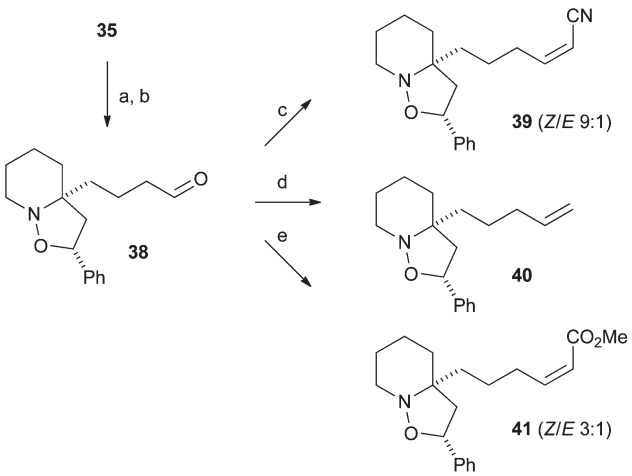
A number of observations are noteworthy. The role of the unsaturated nitrile as a dipolarophile seemed critical in directing cycloaddition to the desired 6,6,5-adduct. Analogous intramolecular dipolar cycloadditions in which the dipolarophile was a TMS-substituted (*Z*)-enyne (*e.g.* **8**, Scheme 1) afforded only the 6,5,5-adduct **10**, and this could not be equilibrated thermally to the (presumably) thermodynamically preferred 6,6,5-adduct.[14]

Model studies: formation of the nitrone by dipolar cycloreversion

In order to overcome the problems of both degradation and side reactions encountered in the above model studies, we protected the intermediate nitrone **26** as a dipolar cycloadduct with styrene. This approach followed that used in our original histrionicotoxin synthesis,[15] and allowed an evaluation of the significance of the *in situ* release of the cyclic nitrone in the presence of the pendant dipolarophile. While Tufariello used a similar protection strategy in his synthesis of (±)-cocaine, employing methyl acrylate to form a cycloadduct,[33,34] our work was, to our knowledge, the first example of styrene adducts as protecting groups for nitrones.

Styrene adduct **35** was prepared using chemistry analogous to that used in the synthesis of hydroxylamine **25**. After coupling the terminal alkyne **31** with iodide **15**, the TBDPS ether **32** was left intact and the benzyl ether was deprotected (boron trichloride–dimethyl sulfide complex)[30] to afford the alcohol. Swern oxidation[27] followed by treatment with hydroxylamine hydrochloride yielded the oxime which was reduced with sodium cyanoborohydride to give hydroxylamine **33**. Heating in toluene (85 °C, 5 h) yielded nitrone **34** which was redissolved in styrene and heated at 80 °C for 16 h in the presence of 2–3 crystals of quinol to inhibit polymerisation. This led to the formation of a 10 : 1 mixture of diastereomeric isoxazolidines **35** and **36** which were separable by flash column chromatography (Scheme 5). The major (*exo*) adduct was converted into the crystalline hydrochloride salt **37** whose structure was confirmed by X-ray crystallography.‡

Deprotection of the silyl ether **35** followed by TPAP oxidation[31] gave the aldehyde **38** which could be converted to the α,β-unsaturated nitrile **39** in good yield under the conditions of Yamamoto (Scheme 6).[29] The α,β-unsaturated nitrile **39** was obtained as a 9 : 1 mixture of *Z*/*E* isomers which were chromatographically inseparable. To provide a basis for comparison the terminal alkene **40** and the α,β-unsaturated ester **41** were also synthesised. Wittig methylenation of the aldehyde **38** yielded the alkene **40** in reasonable yield. A Still–Gennari modification[35] of the Wadsworth–Horner–Emmons reaction[36–40] was carried out with **38** to give a mixture of unsaturated esters containing a 3 : 1 ratio of *Z*/*E* stereoisomers in low yield. The required (*Z*)-isomer **41** was separated chromatographically (Scheme 6).



Scheme 6 Introduction of the pendant dipolarophiles. *Reagents and conditions*: (a) TBAF, THF, 1.5 h, 92%; (b) TPAP, CH_2Cl_2, 20 min, 95%; (c) (i) $TMSCH_2CN$, *n*-BuLi, THF, −78 °C, (ii) $B(O^iPr)_3$, THF, −78 °C, (iii) **38**, THF, −78 °C, 20 min, 76%; (d) (i) $[MePPh_3]^+I^-$, *n*-BuLi, THF, −78 °C, 1 h, (ii) **38**, THF, −78 °C, 30 min, 25 °C, 20 min, 64%; (e) (i) $(CF_3CH_2CO)_2P(O)CH_2COOMe$, KHMDS, THF, −78 °C, 30 min; (ii) **38**, THF, −78 °C, 2 h, 34%.

Table 2 Cycloreversion–cycloaddition reactions of the isoxazolidines **39–41** and product ratios[a]

Alkene	*t*/h	*T*/°C	Products	Ratio	Yield[d] (%)
39[a]	2	100	—	—	0
39[a]	5	160	**29**, **39**	1 : 5	15
39[a]	3	185	**27**, **28**, **29**, **30**	6 : 1 : 25 : 5	41
39[b]	0.5	150[c]	**27**, **28**	6 : 1	92
39[b]	0.25	140[c]	**27–30**, **39**		
39[b]	0.33	140[c]	**27**, **28**	6 : 1	74
40[a]	3	160	**42**	—	72
41[b]	1.6	140[c]	**41**, **43**	1 : 2	95
41[b]	0.5	180[c]	**43**	—	59

[a] Reactions carried out in toluene. [b] Reactions carried out in chlorobenzene. [c] Reaction carried out in microwave. [d] Combined yield of all products.

The various alkenes **39–41** were then subjected to cycloreversion–cycloaddition conditions, and the results are summarised in Table 2.

The α,β-unsaturated nitrile **39** was heated in toluene (sealed tube) in order to induce the formal cycloreversion–cycloaddition. Lower temperatures below 190 °C (Table 2, condition 1 and 2) resulted in either no reaction (at 100 °C) or the formation of **29** in low yield (160 °C) along with degradation and recovery of starting material. The reactions could not be forced to completion; heating for a longer duration led to further degradation and a concomitantly low yield. It was, however, possible to crystallise the adduct **29** from dichloromethane/pentane and the structure was confirmed by X-ray crystallography.‡

‡ Crystallographic data:

Data for 29. $C_{11}H_{16}N_2O$, M = 192.26, orthorhombic, a = 9.6609(9), b = 11.5354(11), c = 9.1360(5) Å, U = 1018.14(15) Å^3, T = 180(2) K, space group $Pca2_1$, Z = 4, μ(Mo Kα) = 0.082 mm^{-1}, 5693 reflections collected, 1705 unique (R_{int} = 0.0974), $R_1[I > 2\sigma(I)]$ = 0.0401, wR_2(all data) = 0.0878, CCDC deposition number 881632.

Data for 37. $C_{33}H_{44}ClNO_2Si$, M = 550.23, triclinic, a = 11.7549(6), b = 13.7175(9), c = 20.1662(13) Å, α = 84.294(3), β = 75.250(4)°, γ = 89.894(4)°, U = 3128.1(3) Å^3, T = 180(2) K, space group $P\bar{1}$, Z = 4, μ(Mo Kα) = 0.189 mm^{-1}, 26 871 reflections collected, 10 742 unique (R_{int} = 0.0510), $R_1[I > 2\sigma(I)]$ = 0.0729, wR_2(all data) = 0.1828, CCDC deposition number 620201.

Data for 48. $C_{19}H_{24}N_2O_2$, M = 312.40, monoclinic, a = 8.4675(15), b = 11.662(2), c = 8.6820(16) Å, β = 103.279(5)°, U = 834.4(3) Å^3, T = 150(2) K, space group $P2_1$, Z = 2, λ = 0.6885 Å (SRS Daresbury), μ = 0.081 mm^{-1}, 7499 reflections collected, 3867 unique (R_{int} = 0.0575), $R_1[I > 2\sigma(I)]$ = 0.0643, wR_2(all data) = 0.1555, CCDC deposition number 885212.

Data for 75. $C_{24}H_{41}NO_2Si$, M = 403.67, monoclinic, a = 7.7031(3), b = 9.8991(6), c = 15.8805(8) Å, β = 98.831(3)°, U = 1196.59(11) Å^3, T = 180(2) K, space group $P2_1$, Z = 2, μ(Mo Kα) = 0.890 mm^{-1}, 7454 reflections collected, 4564 unique (R_{int} = 0.0432), $R_1[I > 2\sigma(I)]$ = 0.0439, wR_2(all data) = 0.1107, CCDC deposition number 865489.

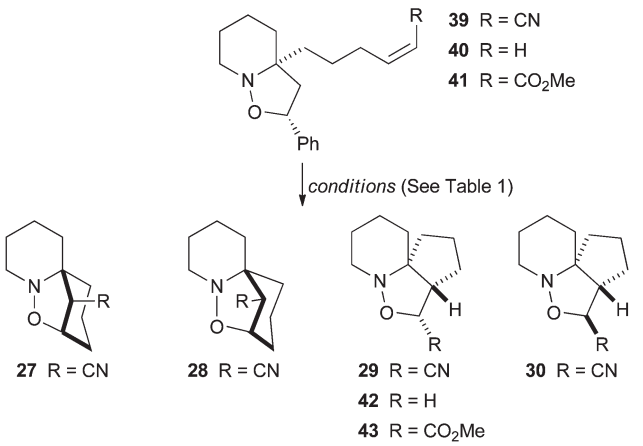
See ESI for further crystallographic data.

An improved reaction outcome was realised under the cycloreversion–cycloaddition conditions used in the histrionicotoxin synthesis[15] (185 °C in degassed toluene in a sealed tube). After heating at 185 °C for 3 h (Table 2, condition 3) flash column chromatography of the reaction mixture afforded pure samples of the two major products, the 6,5,5-adduct **29** and the 6,6,5-adduct **27** and the minor epimeric 6,5,5-adduct **30**. However, it was not possible to separate the minor equatorial cyano-substituted 6,6,5-adduct **28** from the adduct **27**. The ratio of starting materials to products was determined by ^{1}H NMR spectroscopic analysis of the mixtures obtained from the initial column (see Table 1 for characteristic signals). Much greater success was realised using microwave dielectric heating. Microwave heating of the isoxazolidine **39** in chlorobenzene at 150 °C for 30 min resulted in the formation of the 6,6,5-adducts **27** and **28** in unprecedented yield (92%) as a 6 : 1 mixture respectively with no evidence of the 6,5,5-adducts. Microwave irradiation of **39** at 140 °C for 15 min resulted in the formation of the complete range of 6,6,5- and 6,5,5-adducts with some recovered starting material (quantitative mass recovery). However, heating the α,β-unsaturated nitrile **39** at 140 °C for the slightly longer reaction time of 20 min yielded the 6,6,5-adducts **27** and **28** once more as a 6 : 1 mixture (74%). This reaction was followed by TLC analysis and it was possible to observe the initial formation of the 6,5,5-adducts **29** and **30** after 5 min in addition to the 6,6,5-adducts **27** and **28**. After a further 15 min the 6,5,5-adducts were not visible by TLC, having been isomerised to the 6,6,5-adducts **27** and **28**. This indicates that the 6,5,5-adducts appear to be the products of kinetic control and the use of higher temperatures drives the reaction in the direction of the more thermodynamically stable 6,6,5-adducts. This is consistent with both Stockman's and our previous observations.[13,22]

Attempted standard cycloreversion–cycloaddition of the isoxazolidine **40** at 185 °C in toluene (freeze-thaw degassed) in a sealed tube resulted in complete decomposition. However, when the reaction was repeated at 160 °C for 3 h the volatile 6,5,5-adduct **42** was formed in good yield (72%).

Finally, the cycloreversion–cycloaddition reaction of the oxazolidine **41** carrying the (*Z*)-α,β-unsaturated ester was investigated under microwave conditions at 140 °C for 1 h 40 min, and the 6,5,5-adduct **43** was obtained in good yield (62%) in addition to starting material (33%) (Scheme 7). Microwave irradiation at 180 °C for 30 min afforded a similar yield (59%) and starting material was not recovered and TLC analysis indicated that considerable decomposition had occurred.

The formation of 6,6,5-adduct **27** as the major product from the cyano-substituted alkene **39** in these studies reinforces our belief in the importance of the nitrile substituent on the regiochemical outcome of the dipolar cycloaddition. The formation of the 6,5,5-adducts **42** and **43** in dipolar cycloaddition reactions to the nitrones generated by cycloreversion of styrene and involving, respectively, the unsubstituted and terminal methoxycarbonyl-substituted dipolarophiles demonstrates that the regiochemical outcome has not been influenced by the *in situ* cycloreversion process. This is consistent with Stockman's observations.[41–43] The cyano-substituent is the dominating influence on the regioselectivity of the cycloaddition, and the stereospecific thermal interconversion of 6,5,5-adducts to the corresponding 6,6,5-adducts suggests that the stereochemical integrity of the (*Z*)-unsaturated nitrile is maintained throughout these thermodynamically controlled processes.



Scheme 7 Thermal dipolar cycloreversion–cycloaddition of the isoxazolidines **39–41**.

Model studies: histrionicotoxin precursors

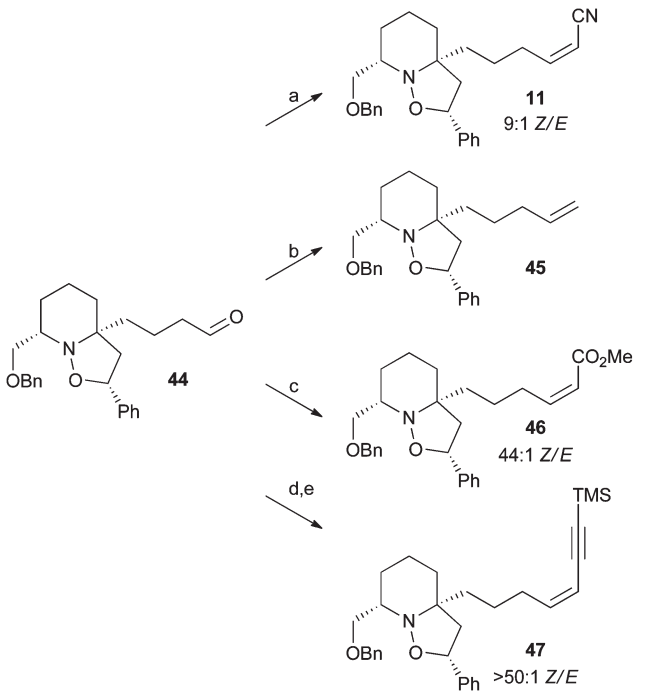
We have proposed in the above model studies that the polarising effect of the cyano substituent on the dipolarophile (together with subtle influences on the LUMO energy of the dipolarophile) are the important factors controlling the regiochemistry of the cycloaddition. These issues are discussed in detail in a computational publication.[20] We now describe analogous studies on benzyloxymethyl-substituted (in the carbocyclic ring) derivatives that are analogues of the isoxazolidine **11** in which the pendant dipolarophile has been systematically varied.

Using enantiomerically-enriched aldehyde **44** as a common precursor, a variety of different double bond forming protocols were utilised (Scheme 8).[14] The α,β-unsaturated nitrile **11** was synthesised in high yield and moderate *Z*/*E* selectivity under the conditions of Yamamoto.[29] Wittig methylenation yielded the terminal alkene **45** in variable yield, while a Still–Gennari reaction[35] was used to synthesise ester **46** with superb stereoselectivity (44 : 1 *Z*/*E*). A Stork–Wittig reaction[44] followed by a Sonogashira coupling[45] with TMS-acetylene afforded the TMS-enyne **47** with excellent stereoselectivity (>50 : 1 *Z*/*E*).

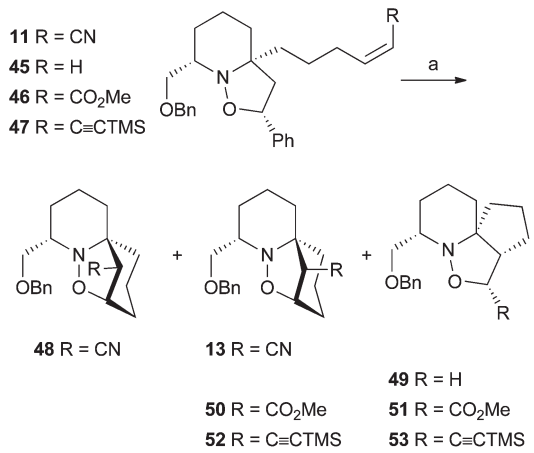
These alkenes were then subjected to the cycloreversion–cycloaddition conditions (toluene, sealed tube, 190 °C) for varying reaction times and the results of these reactions, including that of the α,β-unsaturated nitrile **11** are summarised in Table 3.

Under these conditions, cyano-substituted **11** was converted to the 6,6,5-adduct **13**, with a trace of **48** also detected. The isoxazolidine **45** was converted exclusively to the 6,5,5-adduct **49**. Further heating resulted in total decomposition. The methyl ester **46** was converted to a mixture of the 6,6,5- and 6,5,5-adducts **50** and **51**. Enyne **47** also gave a mixture of products (Scheme 9).

Thermal equilibration of the separated adducts from the cycloaddition–cycloreversion of the enyne **47** was attempted. Heating the 6,6,5-adduct **52** under the standard conditions of 190 °C in toluene (sealed tube) resulted in formation of the 6,5,5-adduct **53**



Scheme 8 Introduction of various pendant dipolarophiles. *Reagents and conditions*: (a) (i) $TMSCH_2CN$, *n*-BuLi, THF, −78 °C, (ii) $B(O^iPr)_3$, (iii) **44**, THF, 84% (9.1 : 1 *Z*/*E*). (b) (i) $[MePPh_3]^+I^-$, *n*-BuLi, THF, −30 °C; (ii) **44**, THF, −78 °C → rt, 50–85%; (c) $(CF_3CH_2O)_2P(O)$-CH_2CO_2Me, KHMDS, 18-crown-6, THF, −78 °C, 70% (44 : 1 *Z*/*E*); (d) (i) $[Ph_3PCH_2I]^+I^-$, *n*-BuLi, THF, 0 °C; (ii) **44**, THF, −85 °C, 76% (92 : 8 *Z*/*E*); (e) TMSC≡CH, CuI (cat.), $Pd(PPh_3)_4$ (cat.), Et_2NH, rt, 100%, (>50 : 1 *Z*/*E*).

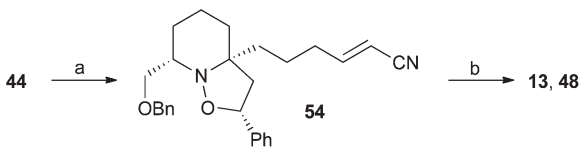


Scheme 9 Thermal dipolar cycloreversion–cycloaddition of the isoxazolidines **11** and **45–47** *Reagents and conditions*: (a) PhMe, sealed tube, 190 °C.

Table 3 Cycloreversion–cycloaddition reactions of the isoxazolidines **11**, **45–47** and product ratios[a]

Alkene	*t*/h	Products	Ratio	Yield[b] (%)
11	3.5	**48**, **13**	9 : 92	89
45	9	**49**	—	100
46	4	**50**, **51**	72 : 28	74
47	3	**47**, **52**, **53**	50 : 11 : 39	85

[a] Reactions monitored by TLC. [b] Combined yield of all products.



Scheme 10 Synthesis of (*E*)-α,β-unsaturated nitrile **54**. *Reagents and conditions*: (a) (i) NaHMDS, $(EtO)_2P(O)CH_2CN$, THF, −78 °C (20 min) → rt (2 h) → −78 °C (1.5 h); (ii) **44**, THF, −78 °C → rt; 15 h, 93% (*Z*/*E* 1 : 2); (b) PhMe, sealed tube, 190 °C, 2 h, 35% **48** and 15% **13**.

and on further heating total decomposition. The 6,5,5-adduct **53** was subjected to the same conditions and underwent complete decomposition.

The (*E*)-isomer **54** was synthesised as an inseparable 1 : 2 mixture of *Z*/*E* isomers by treatment of aldehyde **44** with diethyl cyanomethylphosphonate and base (Scheme 10). Subjection of **54** to heating in toluene in a sealed tube gave the separable diastereomers **13** and **48** in a 1 : 2 ratio. An X-ray crystal structure of the isomer **48** was obtained.‡ This complements the previously reported crystal structure of the debenzylated alcohol arising from **13**.[14]

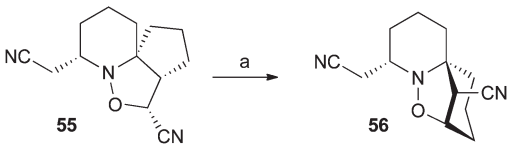
It can be seen from Table 3 that there is a trend in the ratios of products – the increasing π-electron withdrawing nature of the substituent R favouring the 6,6,5-adduct in preference to the alternative 6,5,5-adduct. Another important observation is that the stereochemical integrity of the double bond of the dipolarophile is conserved during the cycloaddition–cycloreversion. We had considered the possibility of a radical or ionic stepwise mechanism as this might have accounted for the apparent change in regioselectivity between the open chain α,β-unsaturated nitrile **25** and styrene adduct **11**. However it is unlikely that either of these mechanistic rationales would account for the conservation of the double bond geometry observed.

In addition to these observations, work published by Stockman[22,23] and Horsley[13] appear to confirm the importance of the cyano substituent.

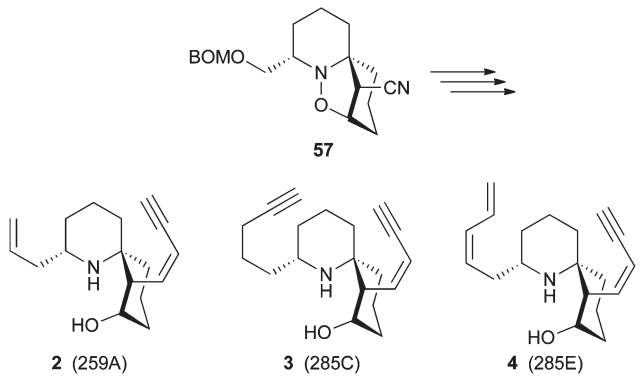
Stockman showed that heating the 6,5,5-isoxazolidine **55** in toluene at 180 °C resulted in the formation of the 6,6,5-adduct **56** as the only observed product (Scheme 11).[22] This seems to be excellent, independent corroboration of our results which have shown the importance of the α,β-unsaturated nitrile. The lack of the styrene protection shows that the styrene present in our work is, in itself, unlikely to play a part in the regiochemical outcome of the cycloaddition.

Synthesis of the "unsymmetrical" histrionicotoxins

The tricyclic adduct **57**, which is the benzyloxymethyl analogue of **13**, is a common precursor for all members of the histrionicotoxin family of alkaloids. The flexibility of our approach is also demonstrated by the conversion of the tricyclic core **57** into three histrionicotoxin derivatives with differing side-chains **2**, **3**



Scheme 11 Stockman's observations. *Reagents and conditions*: (a) PhMe, sealed tube, 180 °C, 95%.



Scheme 12 "Unsymmetrically" substituted histrionicotoxins 259A, 285C and 285E.

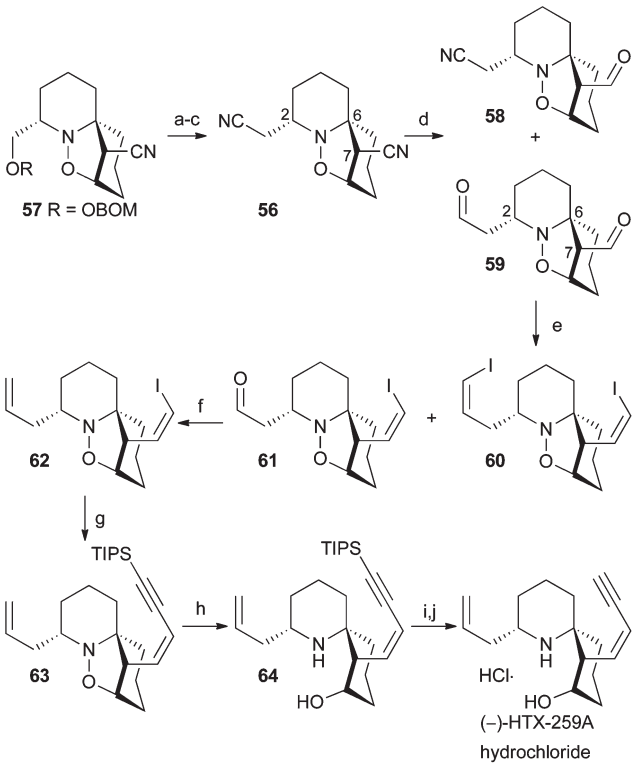
and **4** and further detail of these syntheses is now described (Scheme 12).[17]

Total synthesis of HTX-259A

The syntheses were initiated by an observation during work on the synthesis of parent alkaloid (−)-HTX-283A **1**.[15] During attempts to accomplish a double reduction of dinitrile **56**, obtained in three steps from the tricyclic core **57** (Scheme 13), a difference in the reactivity of the *C*-2 and *C*-7 nitrile functionalities was observed. Exposure of a solution of dinitrile **56** in toluene cooled to −78 °C to 2.5 equivalents of diisobutylaluminium hydride (1.5 M solution in toluene) and quenching after just 15 min furnished a mixture of dialdehyde **59** (59%) and the mono-reduced product **58** (26%) with no recovery of starting material.

Attempts to optimise these results for the chemoselective formation of mono aldehyde **58** were unsuccessful, but on advancing the intermediate one stage further we were able to accomplish chemoselective mono olefination of the dialdehyde **59**. Portionwise addition of the salt free iodophosphonium ylide (1.5 equiv.) to a solution of the dialdehyde **59** in degassed THF at −78 °C yielded the mono-olefinated material **61** in 60% yield together with 10% of the diolefinated material **60** (Scheme 13, step e).

This observed chemoselectivity is remarkable. Molecular modelling (Monte Carlo conformational searching[46] using the MM2 forcefield[47] in MacroModel version 5.5[48]) indicates no obvious difference in steric hindrance of the two aldehyde functionalities and if anything the *C*-7 aldehyde is more sterically hindered than that at *C*-2. Instead, we propose a stereoelectronic rationale for this result. The aldehyde at the *C*-7 position is



Scheme 13 Synthesis of HTX-259A **2**. *Reagents and conditions*: (a) Amberlyst-15®, MeOH, 97%; (b) MsCl, DMAP, NEt_3, CH_2Cl_2, 100%; (c) NaCN, DMSO, 55 °C, 4 days, 69%; (d) DIBAL-H, PhMe, −78 °C, 0.25 h, 59% (**59**), 26% (**58**); (e) (i) $[ICH_2PPh_3]^+I^-$, KHMDS, THF, −30 °C → −78 °C, (ii) **58**, THF, −78 °C, 60% (**61**), 10% (**60**); (f) Cp_2TiMe_2 **61**, PhMe, 110 °C, 1.5 h, 83%; (g) (i) CuI, TIPSC≡CH, $HNEt_2$, rt, (ii) **62**, $Pd(PPh_3)_4$, $HNEt_2$, 17 h, rt, 82%; (h) Zn, AcOH, 0.75 h, 89%; (i) TBAF, THF, 0.75 h: (j) HCl (0.3 M in MeOH), MeOH, 82% (two steps).

conformationally locked in an axial position in a cyclohexane ring with two *trans* diaxial carbon–heteroatom bonds. We propose that owing to overlap of the σ* orbital of the *C*-6 C–N bond with the σ orbital of the aldehyde *C*-7 C–C bond, the *C*-7 aldehyde is more electron deficient than its *C*-2 neighbour and is therefore more electrophilic.

With the mono olefinated material **61** in hand, access to a number of possible "unsymmetrical" histrionicotoxins was available. Initial studies considered one of the simpler alkaloids, (−)-HTX-259A **2**, which possesses an allyl side chain at the *C*-2 position. Installation of the *C*-2 side chain required simply methylenation of the aldehyde. Wittig reagents[49] and more recent modifications[38,50] were believed unsuitable owing to their renowned basicity. As an alternative, the titanium-based reagents developed by Tebbe[51] and Petasis[52] were investigated. Treatment of a solution of the aldehyde **61** and DMAP in THF with a solution of the Tebbe reagent in toluene at −50 °C resulted in complete conversion to the methylenated product **62** in 63% yield. We were delighted to note that this yield could be increased to 83%, upon using the modified Tebbe reagent developed by Petasis. The relatively non-polar product was best separated from the highly polar titanium residues by concentration of the reaction mixture *in vacuo* followed by purification *via* flash

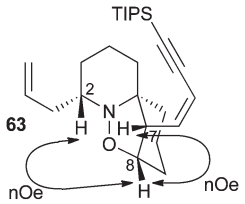


Fig. 3 nOe analysis of **63**.

column chromatography in which the crude residue is pre-absorbed onto silica and dry loaded.

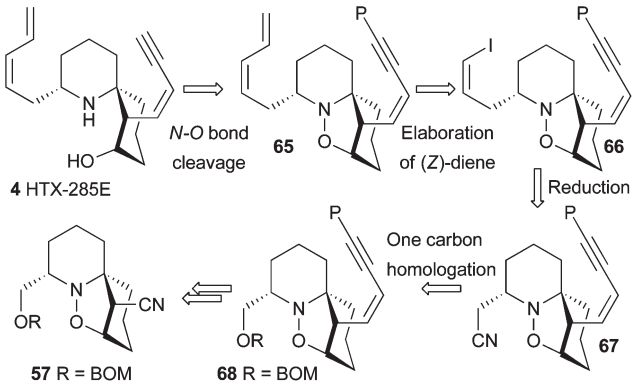
The *C*-7 side chain was completed *via* a Sonogashira coupling[45] with triisopropylsilyl protected acetylene. The stereochemistry of the *C*-2 and *C*-7 stereocentres was confirmed at this stage in the synthesis *via* nOe analysis as shown in Fig. 3.

Reductive cleavage of the isoxazolidine *N–O* bond was accomplished with activated zinc in acetic acid. Careful work up (quenching with saturated aqueous sodium acetate and ethyl acetate with stirring for 1 h, followed by neutralisation and aqueous work up) furnished TIPS-protected histrionicotoxin 259A **64** as a colourless oil in excellent yield (89%). Despite all other spectral properties of this material being as predicted (^{1}H NMR, IR spectroscopy and mass spectrometry) the ^{13}C NMR spectrum lacked any signals relating to sp^2 or sp hybridised carbon atoms. Inspection of the ^{1}H NMR spectrum showed a broadening in the signals corresponding to the protons of the two ring systems. While the reasons for this observation are as yet unclear, a possible explanation is that compound **64** exists as two conformers in dynamic equilibrium and that this effect, combined with a relatively weak sample (3.4 mg), is responsible for the loss of some signals in the ^{13}C NMR spectrum.

Acetylenic deprotection (TBAF) completed the first total synthesis of (−)-HTX-259A **2**. This material was seen to be identical to the natural material on analysis by ^{1}H NMR spectroscopy.[53] However, it was best isolated as its hydrochloride salt. Thus, exposure of a solution of (−)-HTX-259A **2** in dry methanol to a slight excess of methanolic hydrochloric acid (0.3 M) and concentration of the mixture *in vacuo* after 0.5 h stirring furnished (−)-HTX-259A hydrochloride as a colourless oil with the following optical rotation data: $[\alpha]_D^{25.5}$ −54.0 (*c* 0.2 in EtOH). All spectral properties of this material (^{1}H and ^{13}C NMR spectra and mass spectrometry data) were found to be consistent with the proposed structure.

Total synthesis of HTX-285E

Our attention now turned to (−)-histrionicotoxin 285E **4**, which bears a (*Z*)-diene side chain at the *C*-2 position. We envisaged introducing this moiety in a two step procedure – a Stork Wittig olefination furnishing (*Z*)-iodoolefin **66** which could then be submitted to Stille coupling conditions[54] with tributylvinyl tin to furnish the desired (*Z*)-diene (Scheme 14). Owing to the nature of this chemistry, we recognised that it would not be possible to chemoselectively couple to the *C*-2 iodoolefin in the presence of the *C*-7 (*Z*)-iodoolefin. Therefore, it was necessary to introduce the two side chain functionalities independently, and to take advantage of the solo nitrile functionality present in precursor **57**.



Scheme 14 Retrosynthetic analysis of HTX-285E (**4**).

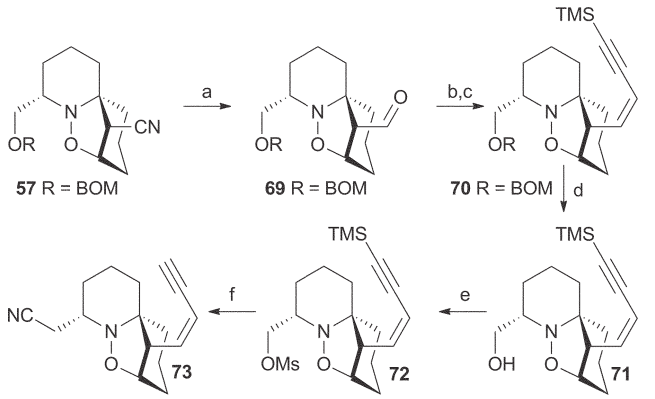
The synthesis thus began by the introduction of the *C*-7 enyne side chain using the two step procedure described previously. Reduction of the nitrile **57** (DIBAL-H at −78 °C in toluene) furnished the aldehyde **69** which was readily converted into the desired (*Z*)-iodoolefin as a single diastereomer on exposure to the modified Stork Wittig conditions.[44] Sonogashira coupling[45] completed the installation of the TMS-protected enyne sidechain to furnish **70** in a 77% overall yield from **69**.

If the *C*-2 side chain was to be installed *via* the two step procedure described in Scheme 14, an initial one carbon homologation was required. Deprotection of the benzyloxymethoxy-protected alcohol **70** employing Amberlyst-15® in methanol and stirring for 17 h initially proceeded in low yield. However, these yields were greatly improved with the addition of excess triethylamine to the methanolic solution after complete consumption of starting alcohol was observed, followed by a further 2–3 h stirring. Removal of the resin *via* filtration and concentration of the filtrate furnished the alcohol **71** in essentially quantitative yield which was then converted to the mesylate **72**. During our previous studies toward the synthesis of (−)-histrionicotoxin, mesylate displacement was found to be slow, requiring 20 equivalents of sodium cyanide in DMSO at 55 °C for 4 days for reasonable conversions to be observed.[14]

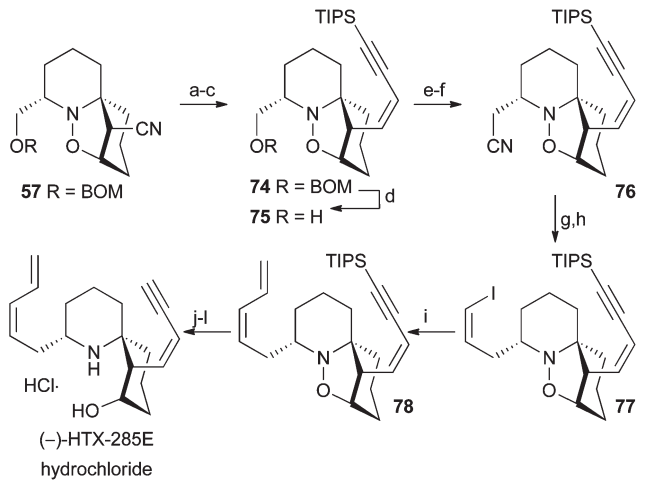
On exposure of the trimethylsilyl protected enyne **72** to these conditions, the desired mesylate displacement occurred in reasonable yield (62%) but was accompanied by removal of the acetylenic trimethylsilyl protecting group (Scheme 15).

Protection of the terminal (*Z*)-enyne was deemed necessary, following problems we had previously experienced during the final stages of our (−)-histrionicotoxin synthesis where we observed reduction of the terminal (*Z*)-enyne side chains during reductive cleavage of the isoxazolidine *N–O* bond.[14] Considering this, we anticipated problems if we carried the terminal (*Z*)-enyne through to the final stages of the synthesis in the deprotected state. Reprotection was considered briefly, but dismissed owing to the base sensitive nature of nitrile **73** and its propensity for β-nitrogen elimination. Therefore, an alternative protecting group was investigated and triisopropylsilyl was found to be suitable.

Sonogashira coupling[45] of **69** this time with triisopropylsilyl acetylene gave the (*Z*)-enyne **74** in quantitative yield (Scheme 16). Longer reaction times were required than for coupling with trimethylsilyl acetylene (17 h *cf.* 1 h). This can be



Scheme 15 Studies toward HTX-285E employing a TMS-protected enyne sidechain. *Reagents and conditions*: (a) DIBAL-H, PhMe, −78 °C, 100%: (b) (i) $[Ph_3PCH_2I]^+I^-$, KHMDS, THF, −30 °C → −78 °C, (ii) **69**, THF, −78 °C, 81%: (c) (i) CuI, TMSC≡CH, $HNEt_2$, rt, (ii) $Pd(PPh_3)_4$, $HNEt_2$, 1 h, rt, 95%: (d) (i) Amberlyst-15®, MeOH, (ii) NEt_3, 99%: (e) MsCl, DMAP, NEt_3, CH_2Cl_2, 85%: (f) NaCN, DMSO, 4 Å MS, 55 °C, 48 h, 62%.



Scheme 16 Synthesis of HTX-285E **4**. *Reagents and conditions*: (a) DIBAL-H, PhMe, −78 °C, 100%; (b) (i) $[Ph_3PCH_2I]^+I^-$, KHMDS, THF, −30 °C → −78 °C, (ii) THF, −78 °C, 81%; (c) (i) CuI, TIPSC≡CH, $HNEt_2$, rt, 5 min, (ii) $Pd(PPh_3)_4$, $HNEt_2$, 17 h, rt, 100%; (d) (i) Amberlyst-15®, MeOH, (ii) NEt_3, 84%; (e) MsCl, DMAP, NEt_3, CH_2Cl_2, 97%; (f) NaCN, DMSO, 4 Å MS, 55 °C, 96 h, 66%; (g) DIBAL-H, PhMe, −78 °C, 100%; (h) $[Ph_3PCH_2I]^+I^-$, KHMDS, THF, −30 °C → −78 °C, (ii) THF, −78 °C, 82%; (i) (i) $PdCl_2(MeCN)_2$, $Bu_3SnCH{=}CH_2$, DMF, 5 min, (ii) NH_4OH (10%), hexane, rt, 17 h, 80%; (j) Zn, AcOH, 0.25 h, 91%; (k) TBAF, THF, 1 h, 84%; (l) HCl (0.3 M in MeOH), MeOH, 84%.

accounted for simply on steric grounds based on the size of triisopropylsilyl *vs.* trimethylsilyl. Acidic deprotection of the benzyloxymethyl ether furnished crystalline alcohol **75** suitable for X-ray analysis,‡ confirming the stereochemistry of the enyne side chain and the absolute configuration.[14]

Mesylation followed by nucleophilic displacement of the mesylate with sodium cyanide now proceeded without problem to yield the one carbon homologated core **76** ready for installation of the *C*-2 side chain.

Diisobutylaluminium hydride-mediated reduction of the nitrile at −78 °C afforded the corresponding aldehyde which, on exposure to the Stork Wittig iodo-olefination[44] conditions described previously, yielded the (*Z*)-iodoolefin **77** in excellent yield (82%) as a single diastereomer by ^{1}H NMR spectroscopy. An efficient sp^2–sp^2 coupling was now accomplished using the protocol described by Stille.[54] Dropwise addition of a solution of tributylvinyl tin in DMF to a rapidly stirred solution of the vinyl iodide **77** and bis(acetonitrile)dichloropalladium(II) in DMF resulted in instantaneous reaction as indicated by a change in colour from orange to black. Initial yields were low (36%), but could be much improved by optimisation of the work-up procedure. Quenching of the reaction mixture with an excess of aqueous ammonia solution and dilution with hexane followed by vigorous stirring overnight resulted in much improved yield. Stirring for shorter periods or without hexane was found to be detrimental to the yields obtained. Using these improved conditions, the (*Z*)-diene **78** was synthesised in excellent yield (80%) with virtually complete retention of alkene configuration as confirmed by ^{1}H NMR spectroscopy.

Completion of the synthesis of (−)-HTX-285E **4** was accomplished as described previously in the synthesis of (−)-HTX-259A **2**. Reductive cleavage of the isoxazolidine *N*–*O* bond was followed by deprotection of the acetylenic silyl protecting group (TBAF, THF, 1 h) furnishing (−)-HTX-285E **4** with an optical rotation of: $[\alpha]_D^{27}$ −23.8 (*c* 0.08 in $CHCl_3$).

The ^{1}H NMR[55] and IR[56] spectra and mass spectrometry data[3] of the synthesised material matched those reported for the natural material. However, the ^{13}C NMR spectrum lacked a number of signals relating to sp and sp^2 carbon atoms. Bearing in mind the similar result obtained during the synthesis of TIPS protected (−)-HTX-259A **64**, variable temperature studies were undertaken to investigate the dynamic equilibration proposed for the azaspiro[5.5]undecan-8-ol core. The ^{1}H NMR spectrum of both TIPS-histrionicotoxin 259A **64** and TIPS-histrionicotoxin 285E show a broadening of the signals corresponding to the protons of the two ring systems. It was hoped that by increasing the temperature of the system, the rate of equilibration between the two conformers would be increased resulting in a sharpening of the signals. Unfortunately however such studies proved inconclusive. Increasing the temperature of the system from 298 K to 328 K showed no change in line broadness. Higher temperatures were not investigated as these preliminary results led us to believe that large temperature changes would be required before any effect was observed.

Stirring a solution of (−)-HTX-285E **4** in methanolic hydrochloric acid gave the corresponding hydrochloride salt with the following optical rotation: $[\alpha]_D^{27.5}$ −38.5 (*c* 0.18 in EtOH), lit.[53] $[\alpha]_D^{25}$ −122 (*c* 1.0 in EtOH). The sample slowly crystallised on standing at −20 °C (mp 231–235 °C). The ^{1}H and ^{13}C NMR spectra were found to be consistent with the proposed structure. An inconsistency was found in the optical rotation in that the measured value was approximately 80° lower than that reported for the natural material. Since the absolute configuration of starting alcohol **75** had been previously conclusively demonstrated *via* X-ray crystallographic analysis, and no evidence of racemisation has been found, the discrepancy is difficult to explain. We do, however, note that the literature value was recorded using a five-fold more concentrated sample.

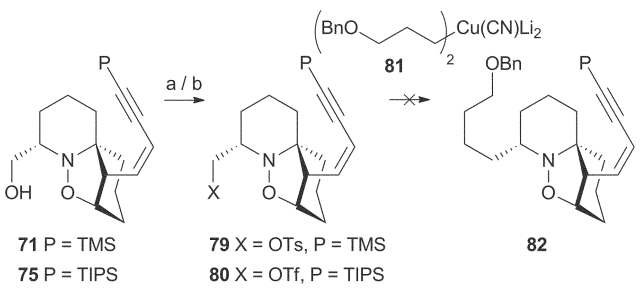
Total synthesis of HTX-285C

A third naturally occurring member of the histrionicotoxin family is (−)-histrionicotoxin 285C. A *C*-19 alkaloid, (−)-histrionicotoxin 285C **3** contains a terminal pentynyl side chain at the *C*-2 position along with the now familiar *C*-7 (*Z*)-enyne.

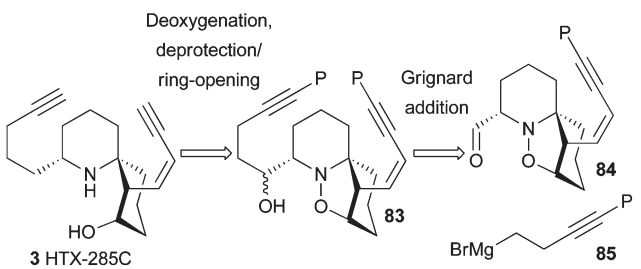
Initial studies depended on the installation of the *C*-2 side chain *via* displacement of a mesylate or tosylate at the *C*-2 position with an alkyl cuprate. However, all attempts were unsuccessful. Cuprate generation from trimethylsilylbutynyl bromide or iodide was found to be impossible. As an alternative, the cuprate of 3-benzyloxy-1-iodopropane (**81**) was formed (the benzyloxy ether functionality acting as a masked alkyne being easily converted *via* deprotection then oxidation followed by the Corey–Fuchs[57] protocol) but all attempts at cuprate displacement of either a tosylate **79** or a triflate **80** at the *C*-2 position failed (Scheme 17). These results are consistent with the earlier results where cyanide displacement of a mesylate substituent at the *C*-2 position required heating at 55 °C for 4 days before reasonable conversion was observed.

Owing to the problems encountered in the nucleophilic displacement studies described above, we considered adding the side chain *via* a nucleophilic addition. Nucleophilic addition of the Grignard reagent **85** to the aldehyde **84** could be followed by deoxygenation completing the side chain installation (Scheme 18).

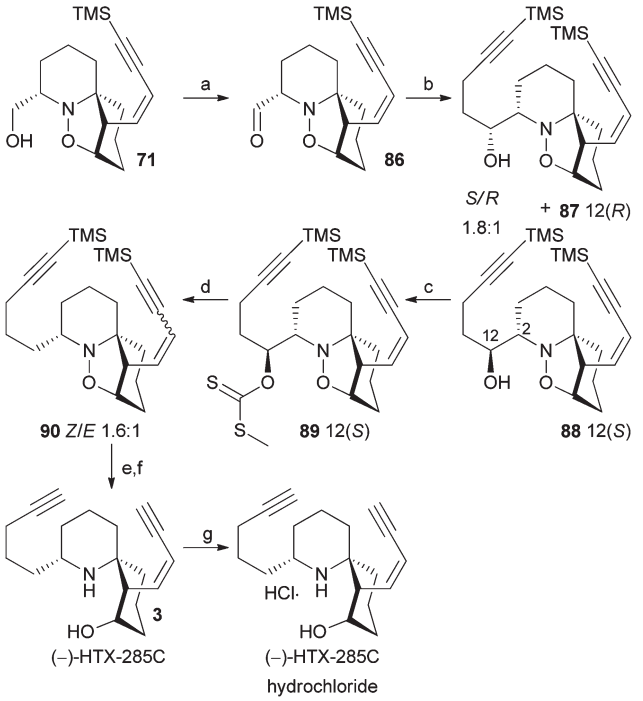
Aldehyde **86** was synthesised from alcohol **71** *via* oxidation with IBX[58] in quantitative yield (Scheme 19). Stork described the synthesis of Grignard reagent **92** from butynyl bromide by the dropwise addition of a solution of 4-trimethylsilyl-3-butynyl-1-bromide **91** in THF to the activated magnesium over a period of 6 h.[59] However, we found that the rate of bromide addition could be increased if the reaction was warmed slightly to 35 °C.



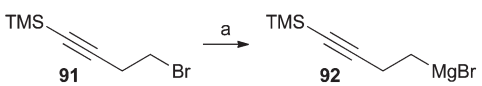
Scheme 17 Attempts to install *C*-2 pentynyl sidechain *via* nucleophilic displacement. *Reagents and conditions*: (a) TsCl, NEt_3, DMAP, CH_2Cl_2, 53% (81% rec.): (b) Tf_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C → rt, 1 h, 69%.



Scheme 18 Retrosynthetic analysis of HTX-285C (**3**).



Scheme 19 Synthesis of HTX-285C, **3**. *Reagents and conditions*: (a) IBX, DMSO, 17 h, 100%; (b) $TMSC{\equiv}C(CH_2)_2MgBr$ **92**, THF, 93% (*S*/*R* 1.8 : 1); (c) (i) **88**, NaH, 0 °C → rt, 1.5 h; (ii) CS_2, 1 h; (iii) MeI, 1.5 h, 83%; (d) Bu_3SnH, AIBN, PhMe, 110 °C, 10 min, 94% (*Z*/*E*, 1.6 : 1); (e) Zn, AcOH, 97%; (f) K_2CO_3, MeOH, 17 h, 88%; (g) HCl (0.3 M in MeOH), 88%.



Scheme 20 Synthesis of Grignard reagent **92**. *Reagents and conditions*: (a) Mg, THF, 22 °C → 35 °C, 0.5 h.

The dropwise addition of a solution of the bromide **91** in THF, to a suspension of activated magnesium in a minimal quantity of THF over a period of 0.5 h was followed by stirring for a further 0.5 h. A halt in effervescence after this time indicated complete formation of the Grignard reagent **92** (Scheme 20).

Dropwise addition *via* cannula of an excess of the Grignard reagent **92** to the aldehyde **86** in THF with rapid stirring, brought about immediate reaction (indicated by a slight warming of the vessel) and furnished the diastereomeric alcohols **88** 12(*S*) and **87** 12(*R*) (1.8 : 1) which could be readily separated by flash column chromatography (Scheme 19).

The assignment of the relative stereochemistry of the two diastereoisomers **87** and **88** was tentatively made by analysis of the ^{1}H NMR coupling constants observed between *H*-2 and *H*-12. Monte Carlo conformational searches[46] using the MM2 forcefield[47] in MacroModel® version 5.5[48] predicted J_{H2-H12} values of 10.0 and 1.9 Hz for the *S* and *R* diastereomers respectively (Fig. 4). By comparison with the observed values of 8.5 and 2.5 Hz for the major and minor diastereomers we can preliminarily assign the major diastereomer to be **88** 12(*S*) and the

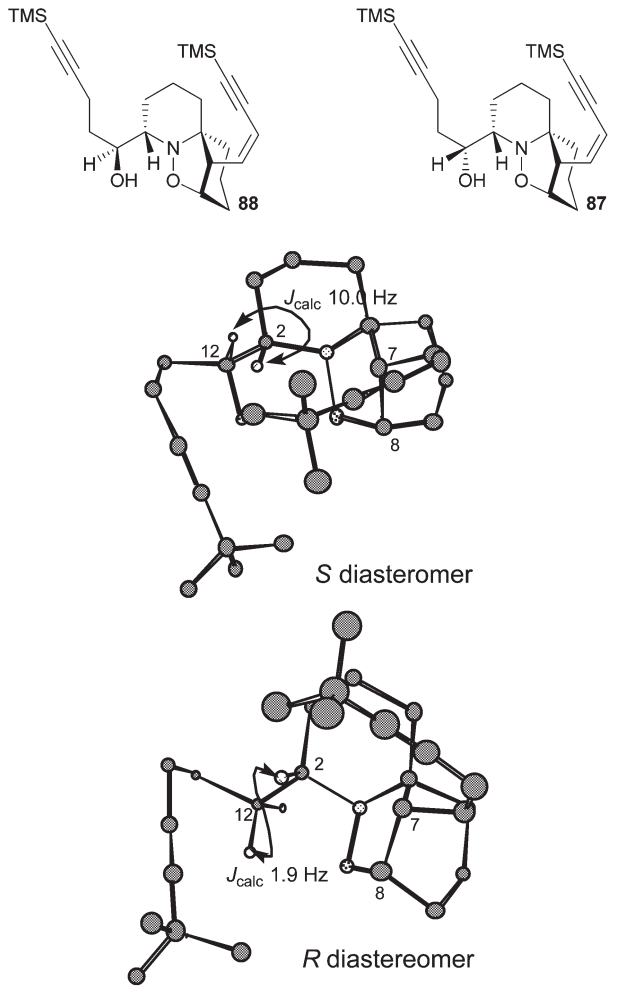


Fig. 4 Monte Carlo conformational models (using MM2 forcefield in MacroModel® version 5.5) of the two diastereoisomers **87** 12(*R*) and **88** 12(*R*).

minor diastereoisomer to be **87** 12(*R*). This is consistent with the Grignard addition having taken place under chelation control.

In order to complete the introduction of the *C*-2 side chain, we were now required to remove the newly formed hydroxy group. We believed this would be best achieved by application of the Barton–McCombie procedure.[60] Alcohol **88** 12(*S*) was converted into the required intermediate xanthate **89** by sequential treatment with sodium hydride, carbon disulfide and methyl iodide. Following Barton's procedure, an excess of tributyltin hydride was added to a solution of xanthate **89** and catalytic AIBN in toluene, and the mixture was heated at 110 °C for 10 min. Concentration *in vacuo* and purification of the crude residue by flash column chromatography afforded two products, the desired deoxygenated material **90** together with its *C*-18 (*E*)-diastereoisomer (Scheme 19).

In an attempt to reduce the amount of isomerisation, the reaction was repeated in the lower boiling solvent, benzene, and heated for exactly 10 min at 80 °C (once addition of tributyltin hydride was complete the reaction was immediately plunged into an oil bath preheated to 80 °C. The vessel was removed from the heat source and plunged into an ice/water bath prior to work-up).

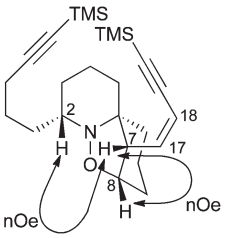


Fig. 5 nOe analysis of **90**.

Although the observed diastereoselectivity was much improved (*Z*/*E* 5 : 1 *cf.* *Z*/*E* 1.6 : 1 in toluene), the yield was lower (70%), resulting in an overall identical net yield of the *Z*-isomer (58%). Epimeric alcohol **87** 12(*R*) was also deoxygenated using the Barton–McCombie conditions described above with identical results to its diastereomer.

The relative stereochemistry was confirmed at this stage *via* nOe analysis as shown in Fig. 5.

Reductive cleavage of the *N*–*O* bond was achieved using the now familiar conditions of zinc in acetic acid, furnishing bis-trimethylsilyl HTX-285C in excellent yield (97%) (Scheme 19). Stirring a solution of bis-trimethylsilyl histrionicotoxin 285C and potassium carbonate in methanol overnight at room temperature afforded (−)-histrionicotoxin 285C **3** [$[\alpha]_D^{18}$ −43.3 (*c* 0.12 in EtOH)] completing the first total synthesis (Scheme 19). The ^{1}H NMR, and IR spectra and mass spectrometry data were all found to be consistent with the proposed structure and identical with those of the natural material.[56,61] Once again however, signals corresponding to sp and sp^2 carbons were not observed in the ^{13}C NMR spectrum. This is contrary to reports in the literature, Tokuyama having been able to assign NMR resonances to all carbon atoms.[53] We have not been able to explain these differences.

In order conclusively to prove the identity of the synthetic sample of (−)-HTX-285C **3**, its hydrochloride salt was prepared. Dropwise addition of anhydrous methanolic hydrochloric acid to a solution of (−)-HTX-285C **3** in anhydrous methanol followed by concentration *in vacuo* furnished (−)-HTX-285C·HCl as a colourless oil which slowly crystallised on standing at −20 °C [$[\alpha]_D^{19}$ −44.6 (*c* 0.12 in EtOH), lit.[62] $[\alpha]_D^{25}$ −43.4 (*c* 1.18 in EtOH), mp 244.5–246.5 °C, lit.[62] 247–250 °C]. ^{1}H NMR, ^{13}C NMR spectra and mass spectrometry data were all found to be consistent with the proposed structure. The ^{13}C NMR spectrum in MeOD showed no evidence of line broadening and all signals were present and correct.

Conclusions

The manuscript describes the importance of the nature of the terminal substituent of the pendant dipolarophile in the intramolecular nitrone dipolar cycloaddition approach to the histrionicotoxin precursors. The model studies show that the *in situ* release of the *N*-alkenyl cyclic nitrone by dipolar cycloreversion of a styrene isoxazolidine adduct allows subsequent intramolecular capture by dipolarophiles to proceed more efficiently than when the nitrone is generated by direct hydroxylamine–alkyne cyclisation.

Further model studies emphasise the importance of the cyano substituent in controlling both the yield and regioselective outcome of the intramolecular dipolar cycloaddition under thermodynamic control, leading preferentially to the required 6,6,5-spirocyclic isoxazolidine cycloadducts.

Application of the knowledge from these model studies enabled these domino cycloreversion–cycloaddition processes to be used to construct the core spirocyclic precursors to the naturally occurring histrionicotoxin alkaloids, HTX-259A (**2**), HTX-285C (**3**) and HTX-285E (**4**).

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