Synthesis and absolute configuration of a constitutionally-new [5.6] spiroacetal from *B. tryoni* (Queensland fruit fly)†

Yvonne K. Booth,[a] Patricia Y. Hayes,[a] Christopher J. Moore,[b] Lynette K. Lambert,[c] William Kitching[a] and James J. De Voss*[a]

Received 6th February 2007, Accepted 12th February 2007
First published as an Advance Article on the web 7th March 2007
DOI: 10.1039/b701833a

A novel spiroacetal, (2*S*,6*R*,8*S*)-2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane (**1**), has been identified from the volatile secretions of female *B. tryoni* by mass spectral analysis and synthesis of an authentic, enantioenriched sample.

Introduction

Spiroacetals are widespread in the insect world,[1] having been identified in the volatile secretions of various species of bees, wasps, beetles and fruit flies.[2] To date, just over 30 constitutionally-different spiroacetals have been characterised, representing five basic ring systems: 1,6-dioxaspiro[4.4]nonanes (**2**), 1,6-dioxaspiro[4.5]decanes (**3**), 1,6-dioxaspiro[4.6]undecanes (**4**), 1,7-dioxaspiro [5.5]undecanes (**5**) and 1,7-dioxaspiro[5.6]dodecanes (**6**) (Fig. 1). Until now, the 1,7-dioxaspiro[5.6]dodecane system (**6**) was represented by a single example, 2-methyl-1,7-dioxaspiro[5.6]dodecane (**7**), identified from the solitary bee *Andrena haemorrhoa*[3] and the fruit fly species *Bactrocera cucumis*,[4] taxonomically close to *B. tryoni*. The constitutionally-new spiroacetal described herein is noteworthy as the second characterised member of system **6** and as the first example of a disubstituted derivative. This work, therefore, enhances the overall landscape of insect-derived spiroacetals.

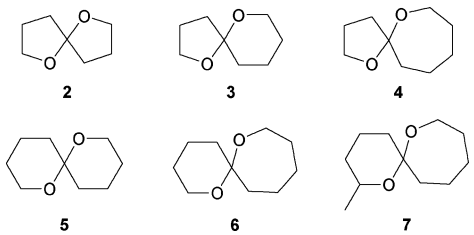


Fig. 1 Spiroacetal ring systems.

We recently reported that female *Bactrocera tryoni* (Frogatt) (Queensland fruit fly), the most destructive horticultural pest in Australia,[5] release a diverse suite of spiroacetals, which ranges from nine to thirteen carbons and includes the presence of unusual even carbon-numbered spiroacetals, as well as the novel branched chain spiroacetal, (2*S*,6*R*,8*S*)–2-ethyl-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane ((2*S*,6*R*,8*S*)-**14**) (Fig. 2).[6] The identification of these spiroacetals was confirmed either by comparison of retention times and co-injection studies with authentic

[a]*Chemistry, School of Molecular and Microbial Sciences, University of Queensland, St. Lucia, Brisbane, Australia 4072. E-mail: j.devoss@uq.edu.au; Fax: +61 (0)7 3365 4299; Tel: +61 (0)7 3365 3825*
[b]*Department of Primary Industries, Yeerongpilly, Brisbane, Australia 4105*
[c]*Centre for Magnetic Resonance, University of Queensland, St. Lucia, Brisbane, Australia 4072*

† Electronic supplementary information (ESI) available: Synthesis of alcohols **16** and **26**, and alkyne **21**. See DOI: 10.1039/b701833a

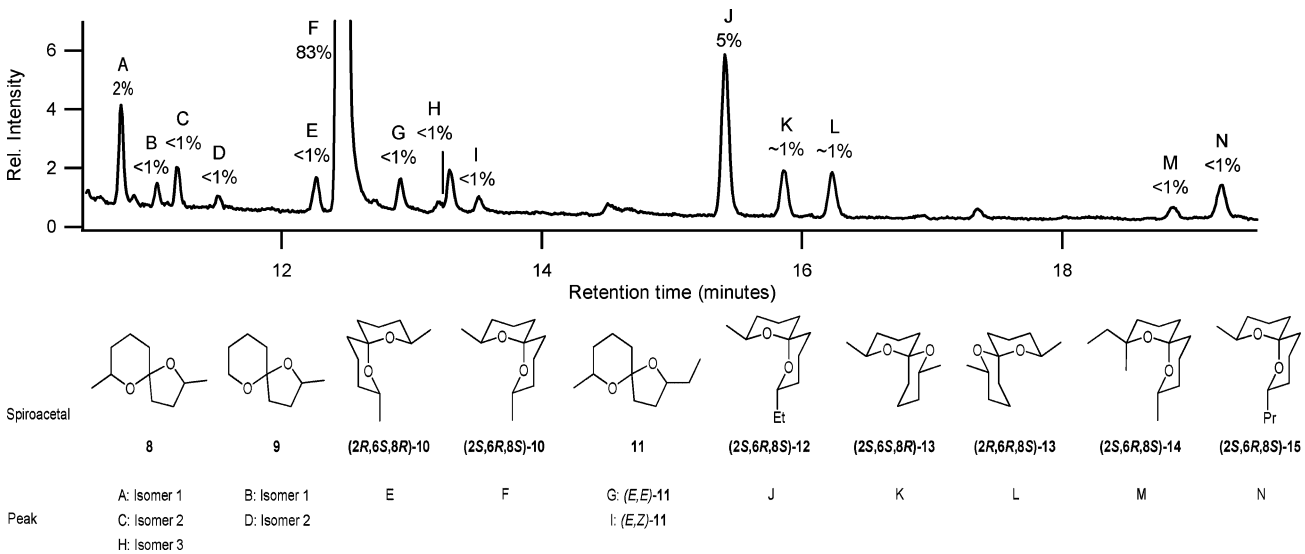


Fig. 2 Enantioselective GCMS of the spiroacetals present in a pentane extract of female *B. tryoni* abdomens. Peak F set to 100 units.

standards,[7] or by comparison with the mass spectra of authentic compounds.[8]

Progress in our ongoing biosynthetic investigations required the identification and characterisation of an exceptionally minor (<0.2% abundance) but biosynthetically-significant spiroacetal component, observed to elute after (2*S*,6*R*,8*S*)-**15** and the previously reported amide, *N*-(3-methylbutyl)acetamide, in the enantioselective GCMS trace (Fig. 3).

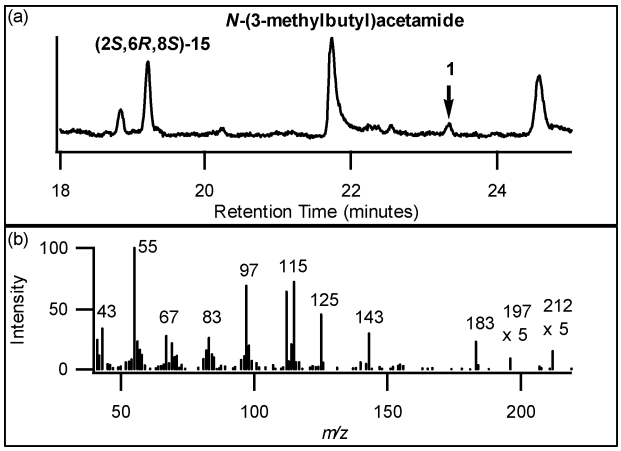


Fig. 3 (a) Enantioselective GC trace of a pentane extract of female *B. tryoni* abdomens, showing newly identified spiroacetal **1**. (b) Mass spectrum of naturally occurring (2*S*,6*R*,8*S*)-**1**.

Results and discussion

As summarised previously,[1] spiroacetals have characteristic fragmentation patterns which guide structural conclusions for unknown compounds. This newly observed spiroacetal, with an apparent molecular ion of 212, has fragment ions at *m*/*z* 197 and 183, corresponding to the loss of methyl and ethyl substituents, respectively. Given this alkyl-substituent pattern, the fragment ions at *m*/*z* 112, 115 and 125 are indicative of a methyl substituted six-membered ring, and the ions at *m*/*z* 140 and 143 are suggestive of an ethyl-substituted seven-membered ring (Fig. 4). Based on this analysis, the component was considered likely to be 2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane (**1**) and, therefore, a constitutionally-new spiroacetal.

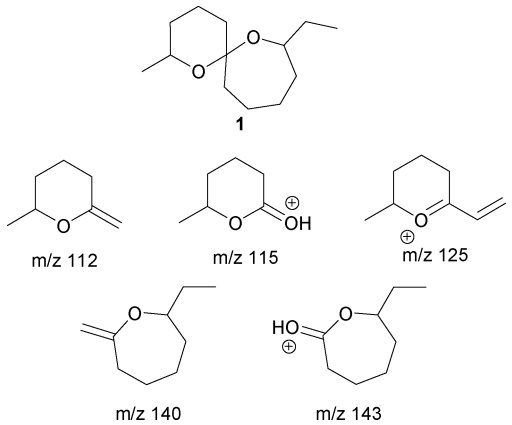
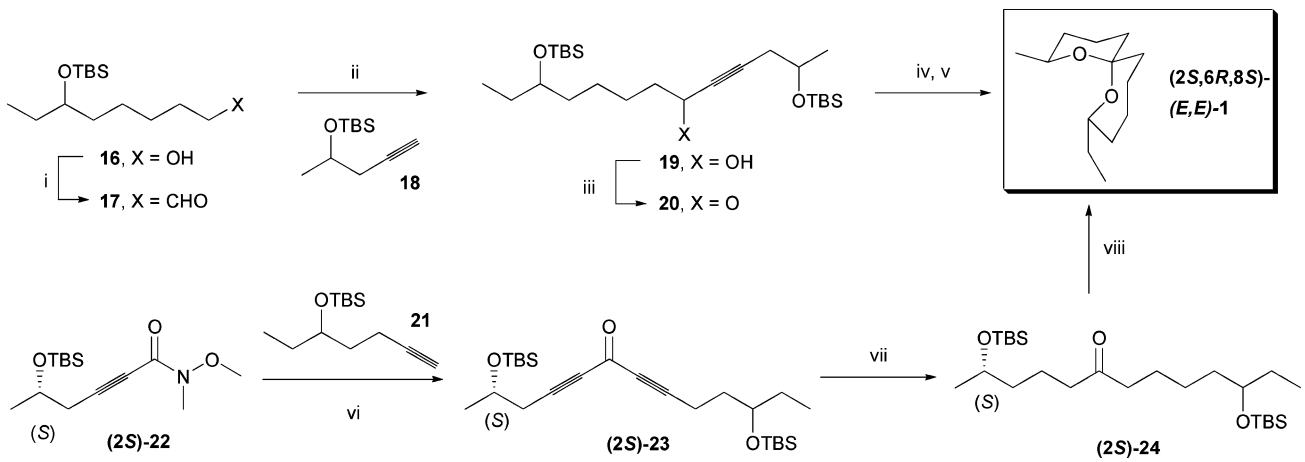


Fig. 4 Mass spectral fragmentation analysis of the newly-identified spiroacetal.

To confirm this deduction, **1** was synthesised in racemic form as outlined in Scheme 1. Oxidation (PCC) of alcohol **16** (synthesised in five steps from commercially available 1,6-hexanediol, see the ESI†), followed by addition of key alkyne **18**[7] to the resulting aldehyde (**17**) furnished propargylic alcohol **19** with the required carbon backbone and oxygenation pattern. Oxidation to ketone **20**, followed by reduction of the triple bond, produced the saturated ketone, ready for deprotection and cyclisation to spiroacetal **1** under acidic conditions. However, even following experimentation with a variety of acidic conditions (75% aqueous acetic acid at 55 °C, trifluoroacetic acid in CH_2Cl_2 and tosic acid in methanol) cyclisation repeatedly proceeded in low yield with the formation of only one isolable diastereomer, which was purified by a combination of flash chromatography and preparative GC. The sole diastereomer was presumed to be the (*E*,*E*)-isomer,‡ with

‡ Substituted spiroacetals are designated (*E*)- when the substituent group and oxygen atom of the alternate ring are on opposite sides of the reference plane (the substituted ring) and (*Z*) when on the same side.



Scheme 1 Synthesis of **1** in both racemic and enantioenriched form. *Reagents and conditions*: (i) PCC, CH_2Cl_2, 83%; (ii) **18**, *n*-BuLi, THF, −78 °C, 59%; (iii) PDC, CH_2Cl_2, 84%; (iv) $H_{2(g)}$, Pd/C, THF, 92%; (v) $TsOH{\cdot}H_2O$, MeOH, 14%; (vi) **21**, MeLi, THF, −40 °C, 84%; (vii) $H_{2(g)}$, Pd/C, Et_3N, EtOAc, 90%; (viii) 75% AcOH, 55 °C, 31%.

maximum anomeric stabilisation from two *axially* directed oxygen atoms (Fig. 5).

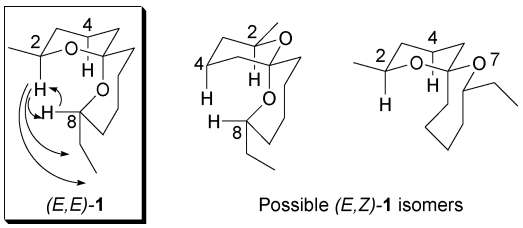


Fig. 5 Observed NOE interactions in (*E*,*E*)-1 and possible (*E*,*Z*)-isomers.

This product bias presumably reflects, under reversible conditions, the free energy differences between the possible isomers. In comparison with [5.5] spiroacetals such as 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**10**) containing only six-membered rings, the additional free energy of the seven-membered ring in **1** is best counteracted in the (*E*,*E*)-manifold. Thus, it is understandable that formation of the (*E*,*E*)-isomer‡ of **1**, with two stabilising anomeric effects (1.4 kcal mol^{-1} each),[9] would be heavily favoured in comparison with the (*E*,*Z*)-isomers, which would possess only one such stabilising effect.

To demonstrate that the sole product was indeed the (*E*,*E*)-isomer as rationalised above, comprehensive NMR analyses were undertaken. Unsurprisingly, the spiroacetal exhibited an extended region of overlapping protons, and 1D-TOCSY experiments were utilised to isolate the two ring systems. Analysis of the multiplet for H-2 (δ 4.00, dqd, J 12.0, 6.3, 2.5 Hz), revealed one large and one small coupling, consistent with an *axial* proton, confirming an *equatorial* orientation for the methyl substituent and a chair conformation for the six membered ring. The H-8 multiplet (δ 3.77, dddd, J 10.3, 6.8, 4.8, 1.1) also displayed one large and one small coupling to ring protons, suggestive of a *pseudo-axial* orientation for the proton, and a *pseudo-equatorial* geometry for the ethyl substituent. In addition, the downfield shifts for both of these protons result from 1,3-*diaxial* interactions with oxygen rather than with carbon,[10] and suggest that the spiroacetal adopts an (*E*,*E*)-configuration.‡ This is supported by the chemical shift of the *axial* H-4 proton (δ 1.95), again indicative of a 1,3-*diaxial* interaction with oxygen (O-7). These analyses effectively eliminate the formation of either of the (*E*,*Z*) isomers‡ (Fig. 5), as H-8 in the first structure and H-4 in the second are 1,3-*diaxial* to a C–C bond and so their chemical shifts would be expected to be upfield of δ 3.77 and δ 1.95, respectively.

Furthermore, the anticipation would be that NOEs would be observed between H-2 and H-8 for the (*E*,*E*) isomer‡ but not for the (*E*,*Z*) isomers,‡ even though the more-flexible seven-membered ring would not adopt the pseudochair conformation as rigidly as the spiroacetal containing a six-membered ring. Indeed, spatial interactions were identified between H-2 and H-8 and also between H-2 and both the methylene and methyl groups of the ethyl substituent (Fig. 5). This confirmed that the synthesised spiroacetal was (*E*,*E*)-**1**, rather than either of the possible (*E*,*Z*)-isomers.

Comparisons of the mass spectral data of synthetic (*E*,*E*)-**1** with that of the natural extract (Fig. 6B and 3B) identified the natural spiroacetal as the novel (*E*,*E*)-2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane, ((*E*,*E*)-**1**). The absolute stereochemistry of (**1**) now required determination.

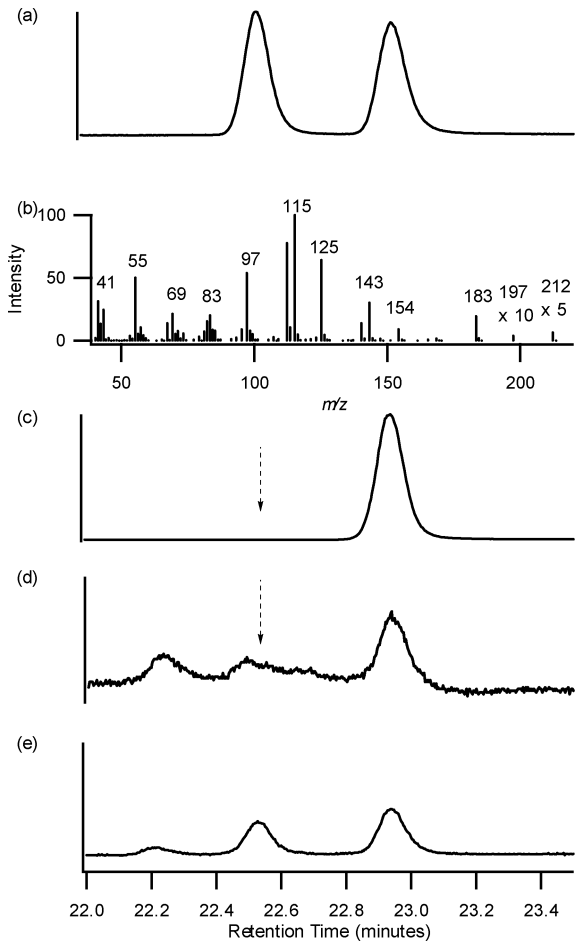
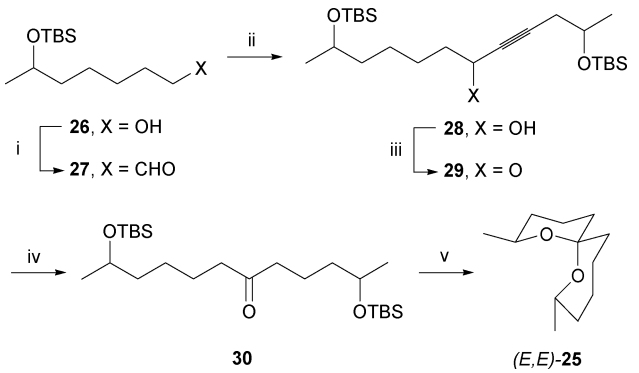


Fig. 6 Total ion current (TIC) of enantioselective GC traces employing single ion monitoring of m/z 212, 183, 143, 125, and 112. (a) Racemic (*E*,*E*)-**1**. (b) Mass spectrum of synthetic **1**. (c) Enantiomerically pure (2*S*,6*R*,8*S*)-**1**. (d) Concentrated pentane extract of female *B. tryoni* abdomens. (e) Co-injection of concentrated extract with racemic (*E*,*E*)-**1**.

Synthesis of enantiomerically pure (*E*,*E*)-**1** employed a previously-developed methodology for the synthesis of enantiopure spiroacetals.[7] Thus, alkyne **21**, synthesised from 4-pentyn-1-ol in three steps (see the ESI†), on addition to key Weinreb amide (2*S*)-**22**[7] provided unsaturated ketone (2*S*)-**23** (Scheme 1). Palladium catalysed reduction of the alkyne moieties, followed by acetic acid-induced cyclisation, delivered only a single isolable enantiomer of (*E*,*E*)-**1** upon purification by flash chromatography. This enantiomer necessarily possessed (2*S*,6*R*,8*S*) stereochemistry and exhibited NMR and mass spectral data identical with those of the racemic product. In agreement with our previous observations on spiroacetal elution orders,[6,11] (2*S*,6*R*,8*S*)-**1** (possessing an (*R*)-spirocentre) was found to elute after (2*R*,6*S*,8*R*)-**1** under our enantioselective GC conditions (Fig. 6A and C). To enable retention time comparisons with the natural extract, single-ion monitoring of several predominant spiroacetal fragmentations (m/z 212, 183, 143, 125, 112) was employed to distinguish the spiroacetals from amide, hydrocarbon and ester components of the extract. From these retention times, (2*S*,6*R*,8*S*)-**1** was identified as the predominant isomer *in vivo* (Fig. 6D). This was

confirmed by co-injection of the natural extract with the racemic standard, which showed a marked increase in intensity for the later-eluting (2*S*,6*R*,8*S*)-isomer in comparison with that of the racemic standard (Fig. 6E, *cf.* A). This absolute stereochemistry is in agreement with determinations for the major spiroacetals found in *B. tryoni* volatiles *viz* (2*S*,6*R*,8*S*)-**10**, (2*S*,6*R*,8*S*)-**12** and (2*S*,6*R*,8*S*)-**15** (Fig. 2,). However, the presence of a low level of (2*R*,6*S*,8*R*)-**1** could not be discounted, and so the enantiomeric excess of the newly identified (2*S*,6*R*,8*S*)-2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane (**1**), although high, is imprecise.

Given the diverse suite of spiroacetals that female *B. tryoni* produce, it was of interest to determine whether the corresponding known C_{11} spiroacetal, 2-methyl-1,7-dioxaspiro[5.6]dodecane (**7**)[12] and the C_{12} spiroacetal 2,8-dimethyl-1,7-dioxaspiro[5.6]dodecane (**25**) were also biosynthesised. As such, (*E*,*E*)-**25** was synthesised in racemic form following an analogous procedure to that used for (*E*,*E*)-**1**, except that the initial alcohol (**26**) was one carbon shorter (Scheme 2). Once again, a low yield of a single isolable diastereomer was obtained after purification by preparative GC.



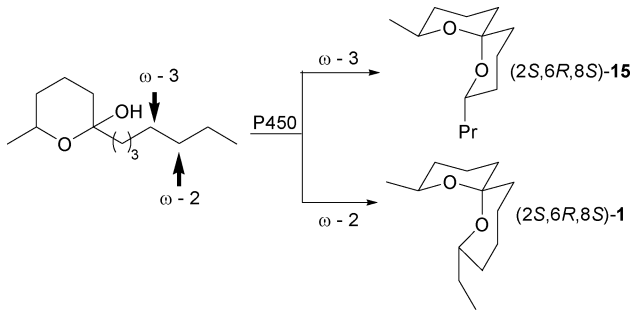
Scheme 2 Synthesis of (*E*,*E*)-**25** in racemic form. *Reagents and conditions*: (i) PCC, CH_2Cl_2, 65%; (ii) alkyne **18**, *n*-BuLi, THF, −78 °C, 78%; (iii) PDC, CH_2Cl_2, 86%; (iv) $H_{2(g)}$, Pd/C, THF, 96%; (v) TsOH·H_2O, MeOH, 19%.

However, thorough scrutiny of the enantioselective GCMS trace of the pentane extract of female abdomens failed to reveal the presence of either spiroacetal **7** or **25**. The clear presence of the C_{13} spiroacetal, (2*S*,6*R*,8*S*)-2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane (**1**), when the corresponding C_{11} and C_{12} spiroacetals are absent (or at vanishingly low concentrations) has interesting biosynthetic implications.

Although the details of the origin of these spiroacetals in *B. tryoni* is still uncertain, it is postulated to involve P450 catalysed oxidation of an alkyltetrahydropyranol precursor as the penultimate biosynthetic step.[13] It is therefore feasible that *B. tryoni* may produce several components of its spiroacetal blend simply by permitting a certain degree of regiochemical flexiblity in this oxidation, in particular with longer chain precursors (Scheme 3). These and related aspects of the biogenesis require studies with suitable, labelled precursors.

Conclusions

In summary, the structure and absolute stereochemistry of a novel spiroacetal from the volatile secretions of *B. tryoni* has been determined to be (2*S*,6*R*,8*S*)-2-methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane (**1**). This new compound is expected to play a pivotal role in determining the biogenesis of spiroacetals in this pestiferous fruit fly species and consequently provide a platform from which we aim to develop a novel inhibition-based control method. The diverse range of spiroacetals produced by *B. tryoni* also makes this fly an ideal model for studying the biosynthesis of this class of compounds in insects.



Scheme 3 Proposed penultimate biosynthetic step of spiroacetal biosynthesis in *B. tryoni*.

Experimental

General methods

All reactions involving moisture or air sensitive reagents were carried out under a nitrogen atmosphere in oven-dried glassware with anhydrous solvents. THF and Et_2O were distilled from sodium and CH_2Cl_2 distilled from calcium hydride. Purifications by flash chromatography were carried out using Scharlau silica gel 60 (230–400 mesh). NMR spectra were recorded on either a Bruker Avance AV500 MHz or AV400 MHz. $CDCl_3$ and C_6D_6 were purchased from Cambridge Isotope Laboratories. ^{1}H NMR spectra were calibrated to 7.24 ppm for residual $CHCl_3$ or 7.15 ppm for C_6D_6. ^{13}C NMR spectra were calibrated from the central triplet peak, to 77.0 ppm for $CDCl_3$ or to 128.0 ppm for C_6D_6. Coupling constants are reported in Hz. GCMS data was recorded on a Shimadzu GC-17A fitted with a DB5 column and attached to either a Shimadzu GCMS-QP5050 or QP5000 detector. The standard temperature program was: 100 °C for two minutes, followed by a 16 °C min^{-1} temperature rise until 250 °C, which was held for ten minutes.

Synthesis of racemic (*E*,*E*)-1

6-(*tert*-Butyldimethylsilyloxy)octanal (17). PCC (1.44 g, 6.68 mmol) was added portionwise to a solution of alcohol **16** (1.13 g, 4.34 mmol, see the ESI†) in dry CH_2Cl_2 (20 mL) and the mixture stirred for two hours. Et_2O (50 mL) was added to dilute the mixture and the brown solution filtered through a celite/silica plug. The organic filtrate was concentrated *in vacuo* and the crude product purified by flash chromatography (1 : 20 EtOAc–hexane) to give aldehyde **17** (0.93 g, 83%) as a colourless oil. Anal. found: C, 65.1; H, 12.0. Calc. for $C_{14}H_{30}O_2Si$: C, 65.1; H, 11.7%; δ_H (500 MHz, $CDCl_3$) 0.00 (3 H, s), 0.01 (3 H, s), 0.82 (3 H, t, *J* 7.4), 0.85 (9 H, s), 1.21–1.47 (6 H, m), 1.60 (2 H, pentet, *J* 7.4), 2.40 (2 H, t, *J* 7.4), 3.55 (1 H, pentet, *J* 5.6), 9.73 (1 H, bs); δ_C (125 MHz, $CDCl_3$) −4.5, −4.4, 9.5, 18.1, 22.3, 24.9, 25.9 (3 C), 29.7, 36.2, 43.9, 73.1, 202.7; *m*/*z* (EI) 257 (M^+-1, 0.1%), 229 (3),

201 (19), 173 (7), 159 (10), 131 (38), 109 (27), 75 (100), 73 (68), 67 (45).

2,11-Bis(*tert*-butyldimethylsilyloxy)tridec-4-yn-6-ol (19). Butyllithium (1.2 M solution in hexanes, 3.0 mL, 3.60 mmol) was added dropwise to a stirred solution of alkyne **18** (0.70 g, 3.53 mmol) in dry THF (15 mL) at −10 °C under nitrogen. The solution was stirred at −10 °C for 30 minutes and aldehyde **17** (0.83 g, 3.21 mmol) in dry THF (6 mL) added slowly by syringe. After stirring for 30 minutes, saturated NH_4Cl solution (20 mL) was added, the organic layer diluted with Et_2O (20 mL) and the layers separated. The aqueous phase was extracted with Et_2O (3 × 20 mL) and the combined organic extracts washed with H_2O (20 mL) and saturated NaCl solution (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (1 : 20 EtOAc–hexane) yielded a diastereomeric mixture of propargylic alcohols **19** (0.86 g, 59%), as a colourless oil. Anal. found: C, 66.1; H, 11.8. Calc. for $C_{25}H_{52}O_3Si_2$: C, 65.7; H, 11.5%; δ_H (500 MHz, $CDCl_3$) 0.012 (6 H, s), 0.044 (3 H, s), 0.051 (3 H, s), 0.83 (3 H, t, *J* 7.4), 0.862 (9 H, s), 0.864 (9 H, s), 1.19 (3 H, d, *J* 6.1), 1.21–1.48 (8 H, m), 1.59–1.68 (2 H, m), 1.70 (1 H, bs), 2.24 (1 H, dddd, *J* 16.4, 7.1, 2.0, 1.1), 2.35 (1 H, ddd, *J* 16.4, 5.6, 1.9), 3.55 (1 H, pentet, *J* 5.6), 3.91 (1 H, sextet, *J* 6.1), 4.30–4.34 (1 H, m); δ_C (125 MHz, $CDCl_3$) −4.76, −4.65, −4.48, −4.44, 9.6, 18.10, 18.15, 23.3, 25.03 and 25.05, 25.41 and 25.43, 25.8 (3C), 25.9 (3C), 29.6, 29.7, 36.4, 38.1, 62.68 and 62.69, 67.62 and 67.63, 73.3, 82.70 and 82.72, 82.76 and 82.78; *m*/*z* (EI) 427 (M^+−29, 0.1%), 399 (0.2), 267 (5), 185 (2), 173 (9), 159 (66), 119 (57), 103 (31), 75 (91), 73 (100).

2,11-Bis(*tert*-butyldimethylsilyloxy)tridec-4-yn-6-one (20). PDC (1.35 g, 3.58 mmol) was added portionwise to a solution of propargylic alcohols **19** (0.82 g, 1.79 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 20 hours. Et_2O (30 mL) was added to dilute the mixture and the solution filtered through a celite plug. The organic filtrate was concentrated *in vacuo* and the crude oil purified by flash chromatography (1 : 20 EtOAc–hexane) to yield propargylic ketone **20** (0.68 g, 84%). Anal. found: C, 66.1; H, 11.4. Calc. for $C_{25}H_{50}O_3Si_2$: C, 66.0; H, 11.1%; δ_H (500 MHz, $CDCl_3$) 0.003 (3 H, s), 0.007 (3 H, s), 0.048 (3 H, s), 0.056 (3 H, s), 0.82 (3 H, t, *J* 7.5), 0.855 (9 H, s), 0.863 (9 H, s), 1.22 (3 H, d, *J* 6.1), 1.20–1.46 (6 H, m), 1.58–1.67 (2 H, m), 2.41 (1 H, dd, *J* 17.0, 6.5), 2.48 (1 H, dd, *J* 17.0, 5.8), 2.50 (2 H, t, *J* 7.5), 3.54 (1 H, pentet, *J* 5.7), 3.99 (1 H, sextet, *J* 6.1); δ_C (125 MHz, $CDCl_3$) −4.85, −4.66, −4.51, −4.45, 9.5, 18.01, 18.12, 23.5, 24.3, 24.7, 25.7 (3 C), 25.9 (3 C), 29.7, 29.8, 36.2, 45.5, 66.9, 73.1, 82.0, 91.3, 188.1; *m*/*z* (EI) 455 (M^+ +1, 0.01%), 425 (0.1), 397 (3), 265 (17), 221 (10), 195 (10), 169 (21), 159 (62), 115 (24), 103 (36), 75 (68), 73 (100).

2,11-Bis(*tert*-butyldimethylsilyloxy)tridecan-6-one (24). Pd/C (60 mg, 10% wt Pd) was added to a stirred solution of propargylic ketone **20** (0.61 g, 1.34 mmol) in dry THF (10 mL) and was subjected to two evacuation/H_2 cycles. The reaction was stirred at room temperature, under an ambient pressure of hydrogen (balloon) for 90 minutes before being filtered through a celite plug and the filtrate concentrated *in vacuo*. The crude product was purified by flash chromatography (1 : 20 EtOAc–hexane) to afford saturated ketone **24** (0.57 g, 92%) as a colourless oil. Anal. found: C, 65.7; H, 12.3. Calc. for $C_{25}H_{54}O_3Si_2$: C, 65.4; H, 11.9%; δ_H (500 MHz, $CDCl_3$) 0.002 (3 H, s), 0.007 (3 H, s), 0.018 (3 H, s), 0.020 (3 H, s), 0.82 (3 H, t, *J* 7.4), 0.86 (18 H, s), 1.09 (3 H, d, *J* 6.1), 1.18–1.67 (12 H, m), 2.359 (2 H, t, *J* 7.5), 2.363 (2 H, t, *J* 7.4), 3.54 (1 H, pentet, *J* 5.7), 3.76 (1 H, sextet, *J* 6.1); δ_C (125 MHz, $CDCl_3$) −4.74, −4.49, −4.44, −4.40, 9.6, 18.10, 18.13, 20.2, 23.7, 24.1, 25.0, 25.88 (3 C), 25.90 (3 C), 29.7, 36.3, 39.1, 42.7, 42.8, 68.3, 73.2, 211.2; *m*/*z* (EI) 457 (M^+-1, 0.1%), 429 (0.04), 401 (5), 269 (16), 227 (10), 185 (19), 173 (18), 159 (15), 145 (25), 95 (19), 75 (100), 73 (66).

2-Methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane ((*E*,*E*)-1). TsOH·H_2O (69 mg, 0.36 mmol) was added to a stirred solution of saturated ketone **24** (80 mg, 0.17 mmol) in MeOH (2 mL), and the reaction stirred at room temperature for 22 hours. H_2O (5 mL) and pentane (5 mL) were added to the mixture and the layers separated. The aqueous phase was extracted with pentane (3 × 5 mL) and the combined organic extracts washed with cold saturated $NaHCO_3$ solution (2 × 10 mL) and saturated NaCl solution (10 mL), dried over $MgSO_4$ and concentrated cautiously *in vacuo* (the rotary evaporator bath was chilled to 5 °C and receiving flask 0 °C). The crude product was purified by flash chromatography (10% CH_2Cl_2 in pentane), and then further purified by preparative GC (Shimadzu GC-9A, OV3 column, isothermal temperature of 150 °C) to give (***E*,*E*)-1** (5 mg, 14%). δ_H (750 MHz, C_6D_6) 0.93 (3 H, t, *J* 7.5), 1.09–1.19 (2 H, m), 1.18 (3 H, d, *J* 6.3), 1.21 (1 H, td, *J* 13.0 and 4.1), 1.33–1.55 (7 H, m), 1.56–1.62 (1 H, m), 1.66–1.70 (1 H, m), 1.83–1.93 (3 H, m), 1.95 (1 H, qt, *J* 13.1 and 3.8), 3.77 (1 H, dddd, *J* 10.3, 6.8, 4.8, 1.1), 4.00 (1 H, dqd, *J* 12.0, 6.3 and 2.5); δ_C (187.5 MHz, C_6D_6) 10.2, 19.8, 22.4, 22.8, 29.7, 30.7, 33.6, 35.1, 35.9, 42.5, 66.8, 71.9, 100.1; *m*/*z* (EI) 212 (M^+, 1%), 197 (0.4), 183 (19), 168 (2), 154 (9), 143 (30), 140 (14), 125 (64), 115 (100), 112 (78), 97 (54), 83 (20), 69 (22), 55 (50), 41 (32); HRMS (ESI) found: 235.1671. Calc. for $C_{13}H_{24}O_2Na$: 235.1674.

Synthesis of enantiomerically pure (*E*,*E*)-(2*S*,6*R*,8*S*)-1

(2*S*)-2,11-Bis(*tert*-butyldimethylsilyloxy)trideca-4,7-diyn-6-one ((2*S*)-23). To a cooled solution (−40 °C) of alkyne **21** (162 mg, 0.7 mmol, see the ESI†) in THF (20 mL) under an inert atmosphere was added dropwise a solution of methyllithium (0.7 M, 1.0 mL). The solution was left stirring for 30 min at the same temperature and then Weinreb amide (**2*S*)-22** (170 mg, 0.6 mmol) in THF (5 mL) was added dropwise. At the end of the addition, the cooling bath was removed and after stirring for another hour at room temperature, the reaction was quenched by addition of a saturated NH_4Cl solution (20 mL). After extraction into ether (3 × 30 mL), the organic layer was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by flash chromatography eluting with 5% ether in hexane to afford (**2*S*)-23** (190 mg, 84%) as a colourless oil. (Found: C, 66.3; H, 10.4. Calc. for $C_{25}H_{46}O_3Si_2$: C, 66.6; H, 10.3%); δ_H (400 MHz, $CDCl_3$) 0.037 (3 H, s), 0.041 (3 H, s), 0.058 (3 H, s), 0.075 (3 H, s), 0.85 (3 H, t, *J* 7.5), 0.87 (18 H, s), 1.22 (3 H, d, *J* 6.1), 1.41–1.50 (2 H, m), 1.61–1.75 (2 H, m), 2.40–2.54 (4 H, m), 3.61 (1 H, quintet, *J* 5.8), 4.01 (1 H, sextet, *J* 6.1); δ_C (100 MHz, $CDCl_3$) −4.68, −4.66, −4.38, 9.2, 15.2, 18.01, 18.06, 23.7, 25.7, 25.8, 29.6, 30.0, 33.9, 66.9, 71.7, 82.2, 83.2, 91.8, 95.0, 161.2; *m*/*z* (EI) 450 ($M^{+\bullet}$−15, 0.01%), 393 (8). 261 (9), 217 (10), 159 (38), 115 (18), 103 (26), 73 (100).

(2*S*)-2,11-Bis(*tert*-butyldimethylsilyloxy)tridecan-6-one ((2*S*)-24). Palladium adsorbed on charcoal (5 mg, 5%) was added to a solution of (**2*S*)-23** (175 mg, 0.4 mmol) in ethyl acetate (5 mL) and triethylamine (0.2 mL). The flask was purged with nitrogen, evacuated and stirred under hydrogen (balloon, 1 atm). The reduction was followed by GC and when complete, the flask was purged with nitrogen and the mixture filtered through a bed of celite then concentrated *in vacuo*. The residue was purified by flash chromatography (5% ether in hexane) to yield saturated ketone (**2*S*)-24** (160 mg, 90%) as a colourless oil. (Found: C, 65.1; H, 12.2. Calc. for $C_{25}H_{54}O_3Si_2$: C, 65.4; H, 11.9%); δ_H (400 MHz, $CDCl_3$) 0.006 (3 H, s), 0.012 (3 H, s), 0.023 (6 H, s), 0.83 (3 H, t, *J* 7.3), 0.86 (18 H, s), 1.09 (3 H, d, *J* 6.0), 1.19–1.69 (12 H, m), 2.37 (4 H, t, *J* 6.6), 3.54 (1 H, quintet, *J* 5.7), 3.76 (1 H, sextet, *J* 6.0); δ_C (100 MHz, $CDCl_3$) −4.7, −4.5, −4.42, −4.38, 9.6, 18.12, 18.15, 20.2, 23.7, 24.1, 25.0, 25.9, 29.7, 36.3, 39.1, 42.8, 42.9, 68.4, 73.2, 211.3; *m*/*z* (EI): 457 ($M^{+\bullet}$ −1, 0.01%), 401 (2), 269 (9), 227 (8), 199 (12), 185 (14), 145 (27), 75 (100).

(2*S*)-2-Methyl-8-ethyl-1,7-dioxaspiro[5.6]dodecane ((*E*,*E*)-(2*S*,6*R*,8*S*)-1). Saturated ketone (**2*S*)-24** (140 mg, 0.3 mmol) was added to an aqueous solution of acetic acid (6 mL, 75%) and heated at 50 °C overnight. The solution was allowed to cool to RT and extracted with pentane (4 × 30 mL). The organic phase was added cautiously to ice cold $NaHCO_3$ (20 mL, sat.) and allowed to stir for 5 min. The pentane layer was separated, dried with $MgSO_4$ and concentrated cautiously *in vacuo* (the rotary evaporator bath was chilled to 5 °C and receiving flask to 0 °C). The crude oil was purified by flash chromatography eluting with pentane to afford (*E*,*E*)-(2*S*,6*R*,8*S*)-1 (20 mg, 31%) as a colourless liquid. Enantiomeric excess estimated by enantioselective GC to be greater than 99.5%. Spectral data was identical to that reported for racemic **1**.

Synthesis of racemic (*E*,*E*)-25

6-(*tert*-Butyldimethylsilyloxy)heptanal (27). PCC (1.31 g, 6.08 mmol) oxidation of alcohol **26** (1.00 g, 4.06 mmol, see the ESI†) in dry CH_2Cl_2 (20 mL) was performed as described for the synthesis of aldehyde **17**. Purification of the crude product by flash chromatography (1 : 20 EtOAc–hexane) afforded aldehyde **27** (0.64 g, 65%) as a colourless oil. Anal. found: C, 63.9; H, 11.8. Calc. for $C_{13}H_{28}O_2Si$: C, 63.9; H, 11.55%; δ_H (500 MHz, $CDCl_3$) 0.00 (3 H, s), 0.01 (3 H s), 0.85 (9 H, s), 1.08 (3 H, d, *J* 6.10), 1.25–1.45 (4 H, m), 1.56–1.65 (2 H, m), 2.40 (2 H, td, *J* 7.4, 1.8), 3.71–3.79 (1 H, m), 9.73 (1 H, t, *J* 1.8); δ_C (125 MHz, $CDCl_3$) −4.8, −4.4, 18.1, 22.1, 23.8, 25.3, 25.9 (3 C), 39.3, 43.9, 68.3, 202.7; *m*/*z* (EI) 243 (M^+-1, 0.1%), 187 (18), 159 (8), 145 (15), 131 (42), 95 (35), 75 (100), 73 (51).

2,11-*bis*(*tert*-Butyldimethylsilyloxy)dodec-4-yn-6-ol (28). Deprotonation of alkyne **18** (0.54 g, 2.72 mmol) with *n*-BuLi (2.27 mL, 1.2 M solution in hexanes, 2.72 mmol) and addition to aldehyde **27** (0.60 g, 2.45 mmol) in anhydrous THF (15 mL), was carried out as described for the preparation of propargylic alcohol **19**. Purification by flash chromatography (1 : 20 EtOAc–hexane) yielded a diastereomeric mixture of propargylic alcohols **28** (0.85 g, 78%) as a colourless oil. Anal. found: C, 65.3; H, 11.8. Calc. for $C_{24}H_{50}O_3Si_2$: C, 65.1; H, 11.4%; δ_H (500 MHz, $CDCl_3$) 0.017 (3 H, s), 0.021 (3 H, s), 0.044 (3 H, s), 0.051 (3 H, s), 0.859 (9 H, s), 0.864 (9 H, s), 1.09 (3 H, d, *J* 6.1), 1.19 (3 H, d, *J* 6.1), 1.21–1.47 (6 H, m), 1.58–1.70 (2 H, m), 2.24 (1 H, dddd, *J* 16.4, 7.1, 1.8, 1.0), 2.35 (1 H, ddd, *J* 16.4, 5.6, 1.9), 3.71–3.78 (1 H, m), 3.91 (1 H, m), 4.32 (1 H, tt, *J* 6.6, 1.9); δ_C (125 MHz, $CDCl_3$) −4.76, −4.72, −4.65, −4.41, 18.10, 18.14, 23.3, 23.8, 25.24 and 25.26, 25.6, 25.80 (3 C), 25.90 (3 C), 29.6, 38.1, 39.6, 62.67 and 62.68, 67.62 and 67.63, 68.49, 82.70 and 82.72, 82.78 and 82.76; *m*/*z* (EI) 442 (M^+, 0.1%), 385 (0.1), 293 (4), 253 (5), 159 (72), 119 (72), 103 (38), 75 (100), 73 (93).

2,11-*bis*(*tert*-Butyldimethylsilyloxy)dodec-4-yn-6-one (29). PDC (1.36 g, 3.62 mmol) oxidation of propargylic alcohols **28** (0.80 g, 1.81 mmol) in dry CH_2Cl_2 (30 mL) was carried out as described for the synthesis of propargylic ketone **20**. Purification by flash chromatography (1 : 20 EtOAc–hexane) gave propargylic ketone **29** (0.68 g, 86%) as a colourless oil. Anal. found: C, 65.1; H, 11.2. Calc. for $C_{24}H_{48}O_3Si_2$: C, 65.4; H, 11.0%; δ_H (500 MHz, $CDCl_3$) 0.006 (3 H, s), 0.012 (3 H, s), 0.046 (3 H, s), 0.054 (3 H, s), 0.85 (9 H, s), 0.86 (9 H, s), 1.08 (3 H, d, *J* 6.1), 1.21 (3 H, d, *J* 6.1), 1.21–1.43 (4 H, m), 1.58–1.67 (2 H, m), 2.41 (1 H, dd, *J* 17.0, 6.5), 2.48 (1 H, dd, *J* 17.0, 5.7), 2.50 (2 H, t, *J* 7.5), 3.71–3.78 (1 H, m), 3.91 (1 H, sextet, *J* 6.1); δ_C (125 MHz, $CDCl_3$) −4.85, −4.76, −4.66, −4.42, 18.01, 18.10, 23.5, 23.8, 24.1, 25.1, 25.7 (3 C), 25.9 (3 C), 29.8, 39.3, 45.5, 66.9, 68.3, 82.0, 91.2, 188.1; *m*/*z* (EI) 441 (M^+ +1, 0.02%), 383 (3), 251 (11), 207 (14), 169 (22), 159 (90), 115 (23), 103 (43), 75 (71), 73 (100).

2,11-*bis*(*tert*-Butyldimethylsilyloxy)dodecan-6-one (30). Hydrogenation of propargylic ketone **29** (0.61 g, 1.38 mmol) with Pd/C (60 mg, 10% wt Pd) and hydrogen gas in THF (10 mL) as described for the synthesis of **24**, afforded saturated ketone **30** (0.59 g, 96%) as a colourless oil, after purification by flash chromatography (1 : 20 EtOAc–hexane). Anal. found: C, 65.0; H, 12.0. Calc. for $C_{24}H_{52}O_3Si_2$: C, 64.8; H, 11.8%; δ_H (500 MHz, $CDCl_3$) 0.009 (3 H, s), 0.016 (3 H, s), 0.019 (3 H, s), 0.022 (3 H, s), 0.85 (9 H, s), 0.86 (9 H, s), 1.08 (3 H, d, *J* 6.0), 1.09 (3 H, d, *J* 6.1), 1.20–1.45 (6 H, m), 1.47–1.66 (4 H, m), 2.360 (2 H, t, *J* 7.5), 2.364 (2 H, t, *J* 7.4), 3.71–3.80 (2 H, m); δ_C (125 MHz, $CDCl_3$) −4.7 (2 C), −4.4 (2 C), 18.10, 18.12, 20.2, 23.7, 23.8, 23.9, 25.4, 25.9 (6 C), 39.1, 39.4, 42.7, 42.8, 68.3, 68.4, 211.2; *m*/*z* (EI) 443 (M^+-1, 0.03%), 387 (5), 255 (12), 213 (13), 199 (15), 185 (21), 163 (22), 145 (35), 119 (20), 75(100), 73 (69).

2,8-Dimethyl-1,7-dioxaspiro[5,6]dodecane ((*E*,*E*)-25). TsOH·H_2O (0.22 g, 1.16 mmol) was added to a stirred solution of saturated ketone **30** (0.25 g, 0.56 mmol) in anhydrous MeOH (3 mL), and the reaction stirred at room temperature for 22 hours. The mixture was diluted by the addition of pentane (10 mL), and solid $NaHCO_3$ added to basify the solution (pH 9). After the addition of H_2O (15 mL), the two phases were separated and the aqueous phase was extracted with pentane (3 × 15 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$ and concentrated cautiously *in vacuo* (the rotary evaporator bath was chilled to 5 °C and receiving flask 0 °C). The crude residue was purified by preparative GC (Shimadzu GC-9A, OV3 column, isothermal temperature of 150 °C) to afford (***E*,*E*)-25** (21 mg, 19%) as the only isolable product. δ_H (500 MHz, C_6D_6) 1.07–1.23 (3 H, m), 1.15 (3 H, d, *J* 6.3), 1.18 (3 H, d, *J* 6.3), 1.32–1.47 (6 H, m), 1.59–1.67 (1 H, m), 1.82–1.91 (3 H, m), 1.97 (1 H, qt, *J* 13.3, 3.9), 3.91–3.99 (2 H, m); δ_C (125 MHz, C_6D_6) 19.7, 22.4,

22.7, 23.6, 29.9, 33.6, 35.7, 38.2, 42.7, 66.77, 66.82, 100.0; *m/z* (EI) 198 (M^+, 3%), 183 (4), 154 (8), 129 (39), 126 (25), 125 (42), 115 (69), 112 (78), 111 (40), 97 (43), 83 (50), 69 (74), 55 (100), 41 (93); HRMS (ESI) found: 221.1518. Calc. for $C_{12}H_{22}O_2Na$: 221.1517.

Mass spectral data of 2-methyl-1,7-dioxaspiro[5.6]dodecane (7). *m/z* (EI) 184 (M^+, 10%), 169 (0.6), 154 (1), 140 (5), 125 (52), 115 (93), 112 (100), 97 (67), 83 (23), 73 (36), 69 (89), 55 (97), 41 (91)

Chiral GCMS conditions

Chiral GCMS analyses were performed on a Shimadzu GC-17A, with a Shimadzu GCMS-QP5050 detector, using a 25 m β-cyclodextrin column (0.22 mm internal diameter) at a column pressure of 92.7 kPa and total flow of 8.7 ml min^{-1}.

Temperature program: 40 °C for 2 minutes, 5 °C min^{-1} until 120 °C; 1 °C min^{-1} until 180 °C.

MS Parameters: single ion monitoring (SIM) *m/z* 212, 183, 143, 125, 112.

B. *tryoni* abdominal extracts

Female *B. tryoni* were laboratory reared on a standard artificial diet of protein hydrolysate, sugar and water for 10–14 days post emergence. The abdominal tips of 150 flies were then removed by dissection and soaked in pentane (~1 mL). The extract was analysed under chiral GCMS conditions.

Mass spectral data of *B. tryoni* spiroacetals

2,7-Dimethyl-1,6-dioxaspiro[4.5]decane (8, isomer 1). *m/z* (EI) 170 (M^+, 4%), 155 (4), 126 (15), 115 (14), 111 (7), 101 (100), 100 (27), 99 (13), 98 (87), 83 (30), 69 (15), 57 (24), 55 (59), 43 (83), 41 (41).

2-Methyl-1,6-dioxaspiro[4.5]decane (9, isomer 1). *m/z* (EI) 156 (M^+, 2%), 141 (1), 128 (3), 112 (10), 101 (74), 100 (26), 98 (34), 83 (24), 55 (41), 43 (100), 41 (57).

2,7-Dimethyl-1,6-dioxaspiro[4.5]decane (8, isomer 2). *m/z* (EI) 170 (M^+, 6%), 155 (4), 126 (9), 115 (9), 111 (7), 101 (100), 100 (22), 99 (9), 98 (76), 83 (27), 69 (16), 57 (28), 55 (54), 43 (98), 41 (56).

2-Methyl-1,6-dioxaspiro[4.5]decane (9, isomer 2). *m/z* (EI) 156 (M^+, 5%), 141 (3), 128 (4), 112 (9), 101 (100), 100 (42), 98 (43), 83 (34), 55 (50), 43 (81), 41 (55).

(*E*,*E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane ((2*R*,6*S*,8*R*)-10 and (2*S*,6*R*,8*S*)-10). *m/z* (EI) 184 (M^+, 12%), 169 (2), 140 (14), 125 (7), 115 (91), 114 (44), 112 (100), 97 (68), 83 (20), 69 (41), 55 (58), 43 (46), 41 (35).

(*E*,*E*)-2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane ((*E*,*E*)-11). *m/z* (EI) 184 (M^+, 5%), 169 (3), 155 (27), 140 (10), 126 (7), 115 (100), 114 (33), 112 (59), 97 (66), 85 (35), 83 (23), 69 (33), 57 (42), 55 (65), 43 (83), 42 (57), 41 (58).

2,7-Dimethyl-1,6-dioxaspiro[4.5]decane (8, isomer 3). *m/z* (EI) 170 (M^+, 20%), 155 (3), 125 (8), 115 (21), 112 (20), 101 (86), 100 (30), 98 (100), 83 (48), 70 (23), 69 (23), 56 (39), 55 (76), 43 (22), 42 (22), 41 (48).

(*E*,*Z*)-2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane ((*E*,*Z*)-11). *m/z* (EI) 184 (M^+, 9%), 169 (3), 155 (38), 140 (11), 126 (7), 115 (100), 114 (27), 112 (69), 97 (77), 85 (43), 83 (31), 69 (52), 57 (40), 55 (91), 43 (70), 42 (49), 41 (54).

(*E*,*E*)-2-Ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane ((2*S*,6*R*,8*S*)-12). *m/z* (EI) 198 (M^+, 19%), 183 (2), 169 (16), 154 (10), 140 (17), 129 (52), 128 (23), 126 (34), 115 (100), 114 (35), 112 (84), 111(42), 97 (65), 83 (52), 69 (55), 68(45), 55 (98), 43 (64), 41 (72).

(*E*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane ((2*S*,6*S*,8*R*)-13 and (2*R*,6*R*,8*S*)-13). *m/z* (EI) 184 (M^+, 7%), 169 (3), 140 (4), 125 (5), 115 (100), 114 (34), 112 (40), 97 (78), 83 (10), 73 (17), 69 (45), 55 (44), 43 (44), 42 (26), 41 (39).

(2*S*,6*R*,8*S*)-2-Ethyl-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane ((2*S*,6*R*,8*S*)-14). *m/z* (EI) 212 (M^+, 2), 197 (3), 183 (13), 143 (15), 142 (11), 140 (8), 125 (37), 115 (21), 112 (33), 97 (19), 83 (29), 55 (93), 43 (100).

(*E*,*E*)-2-Methyl-8-propyl-1,7-dioxaspiro[5.5]undecane ((2*S*,6*R*,8*S*)-15). *m/z* (EI) 212 (M^+, 13), 169 (16), 143 (34), 140 (39), 140 (8), 125 (38), 115 (21), 114 (26), 112 (81), 97 (87), 83 (26), 69 (36), 55 (99), 43 (88), 42 (52), 41 (75).

Acknowledgements

The authors are grateful for IPRS scholarship support for Y. Booth, to Thelma Peek (Department of Primary Industries) for the supply of fruit fly pupae and to Mary Fletcher and Brett Schwartz for GCMS data from an extract of concentrated female *B. tryoni* abdomens.

References

1 W. Francke and W. Kitching, *Curr. Org. Chem.*, 2001, 233–251.
2 M. T. Fletcher and W. Kitching, *Chem. Rev.*, 1995, **95**, 789–828.
3 W. Francke, W. Reith, G. Bergstroem and J. Tengoe, *Z. Naturforsch., Teil C*, 1981, **36**, 928–932.
4 W. Kitching, J. A. Lewis, M. T. Fletcher, R. A. I. Drew, C. J. Moore and W. Francke, *J. Chem. Soc., Chem. Commun.*, 1986, 853–854; W. Kitching, J. A. Lewis, M. V. Perkins, R. Drew, C. J. Moore, V. Schurig, W. A. Konig and W. Francke, *J. Org. Chem.*, 1989, **54**, 3893–3902.
5 R. A. I. Drew, G. H. S. Hooper and M. A. Bateman, *Economic Fruit Flies of the South Pacific Region*, 2nd edn, Department of Primary Industries, Brisbane, 1982.
6 Y. K. Booth, B. D. Schwartz, M. T. Fletcher, L. K. Lambert, W. Kitching and J. J. De Voss, *Chem. Commun.*, 2006, 3975–3977.
7 B. D. Schwartz, P. Y. Hayes, W. Kitching and J. J. De Voss, *J. Org. Chem.*, 2005, **70**, 3054–3065.
8 J. A. Lewis, PhD thesis, University of Queensland, 1987.
9 P. Deslongchamps, *Stereoelectronic Effects in Organic Chemistry*, Pergamon Press, New York, 1983.
10 Y. Q. Tu, A. Hubener, H. Zhang, C. J. Moore, M. T. Fletcher, P. Hayes, K. Dettner, W. Francke, C. S. P. McErlean and W. Kitching, *Synthesis*, 2000, 1956–1978.
11 C. S. P. McErlean, M. T. Fletcher, B. J. Wood, J. J. De Voss and W. Kitching, *Org. Lett.*, 2002, **4**, 2775–2778; C. S. P. McErlean, PhD thesis, University of Queensland, 2002.
12 R. Ballini and M. Petrini, *J. Chem. Soc., Perkin Trans. 1*, 1992, 3159–3160.
13 J. E. Stok, C.-S. Lang, B. D. Schwartz, M. T. Fletcher, W. Kitching and J. J. De Voss, *Org. Lett.*, 2001, **3**, 397–400; M. T. Fletcher, B. E. Mazomenos, J. H. Georgakopoulos, M. A. Konstantopoulou, B. J. Wood, J. J. De Voss and W. Kitching, *Chem. Commun.*, 2002, 1302–1303.