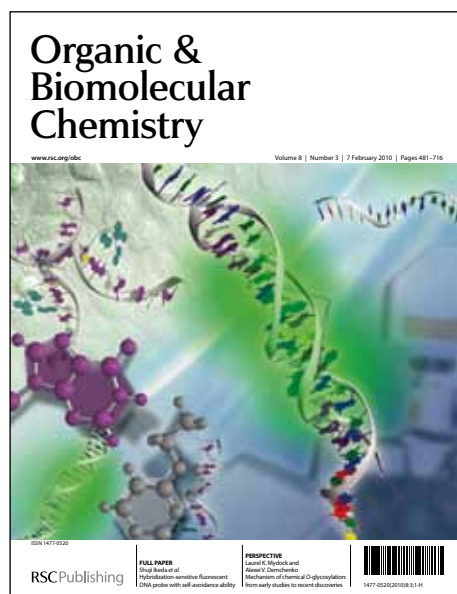


Organic & Biomolecular Chemistry

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard [Terms & Conditions](#) and the [ethical guidelines](#) that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

PAPER

Ethnylbenzenoid metabolites of *Antrodia camphorata*: synthesis and inhibition of TNF expression

Marco Buccini,^a Kathryn A. Punch,^a Belinda Kaskow,^a Gavin R. Flematti,^a Brian W. Skelton,^b Lawrence J. Abraham^a and Matthew J. Piggott^{*a}

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

An improved synthesis of the anti-inflammatory natural product antrocamphin A (**2**), involving a key Castro-Stephens reaction, is presented, along with the first total synthesis of its congener antrocamphin B (**3**). Approaches towards the more complex co-metabolite antrodioxolanone (**4**) were unsuccessful, but a samarium diiodide-mediate pinacol coupling of antrocamphin B did provide the chiral epimers (**51**). Antrocamphin A (**2**) inhibits Tumour Necrosis Factor (TNF) reporter gene expression, but its development as an anti-inflammatory agent may be limited by cytotoxicity.

Introduction

We recently questioned the identity of a natural product isolated from the fungus *Antrodia camphorata* (also called *Taiwanofungus camphoratus*, niu-chang-chih or jang-jy), a commercially important traditional Chinese medicine, which is becoming increasingly rare in its native Taiwan. The proposed structure **1**¹ (Figure 1) made this the first reported example of a naturally occurring acid chloride, which seemed incompatible with a physiological existence, or the extraction and isolation procedure. Indeed, synthetic **1** is highly unstable and rapidly hydrolyses on exposure to atmospheric moisture, and trace water in CDCl₃, making it difficult to characterise.² Moreover, the spectroscopic and mass spectrometric properties of **1** do not match those of the natural product.² These incongruities led us to consider the novel co-metabolites **2–4** reported in the same paper (Figure 1).¹

Antrocamphin A (**2**) has attracted significant attention due to its anti-inflammatory activity,^{1, 3, 4} which is comparable to that of ibuprofen in some assays. Indeed, during the course of our work, Chang, Wu and colleagues reported the first synthesis of **2**, along with a series of analogues that were evaluated for anti-inflammatory activities.⁵ Syntheses of the congener antrocamphin B (**3**), and the symmetrical, dimeric antrodioxolanone (**4**) have not previously been reported.

Herein we describe the first synthesis of antrocamphin B (**3**), an improved synthesis of antrocamphin A (**2**), and approaches towards antrodioxolanone (**4**), culminating in the synthesis of its chiral epimers. Studies aimed at elucidating the mode of anti-inflammatory action of antrocamphin A are also reported.

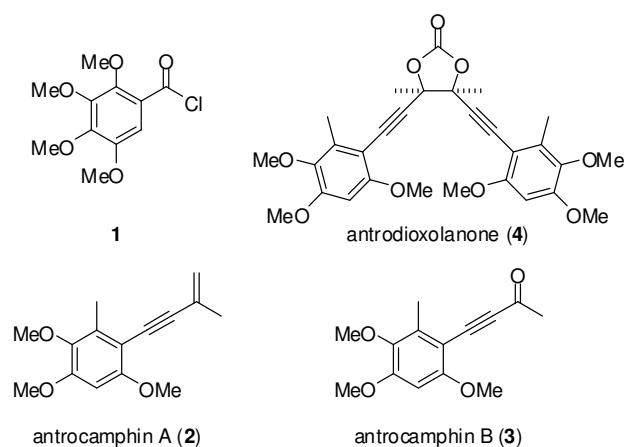
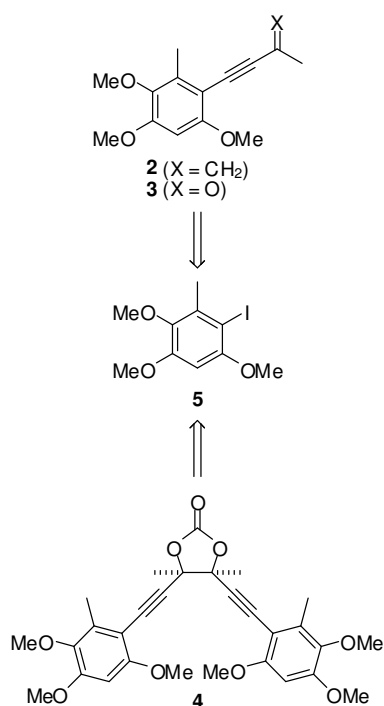


Fig. 1 Benzenoid metabolites reportedly isolated from *Antrodia camphorata*.¹

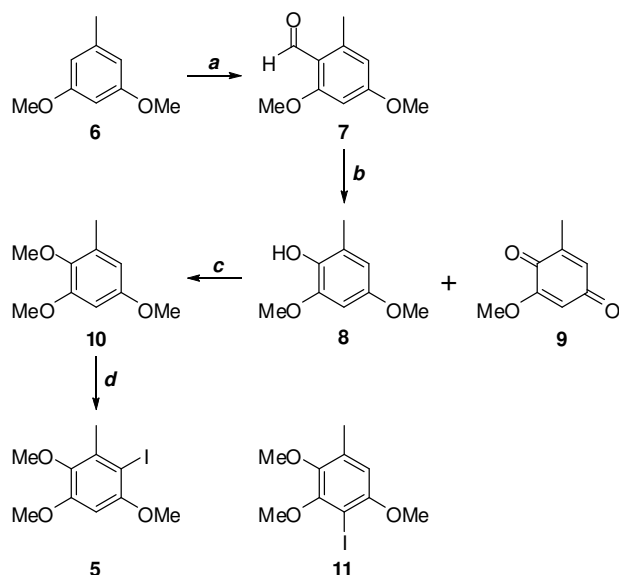
Results and Discussion

The antrocamphins (**2–3**) and antrodioxolanone (**4**) possess a common benzenoid moiety, which we felt could be exploited in our synthetic endeavours. Thus, a simple retrosynthetic analysis led back to Sonogashira reactions of the iodide **5** (Scheme 1). Chang, Wu and co-workers used a similar approach in their synthesis of antrocamphin A, in which they prepared **5** by the silver trifluoroacetate-mediated iodination of 2,3,5-trimethoxytoluene (**10**) (see Scheme 2 for structure), in turn derived from *o*-vanillin in four steps and 46% overall yield.⁵ Our approach began with Vilsmeier-Haack formylation of 3,5-dimethoxytoluene (**6**),⁶ followed by Baeyer-Villiger oxidation⁷ of the resultant benzaldehyde **7** (Scheme 2). Initially the latter reaction provided the desired phenol **8** contaminated by the corresponding quinone **9**, resulting from over oxidation. Although easily separable, the formation of the quinone could be



Scheme 1 Retrosynthetic analysis

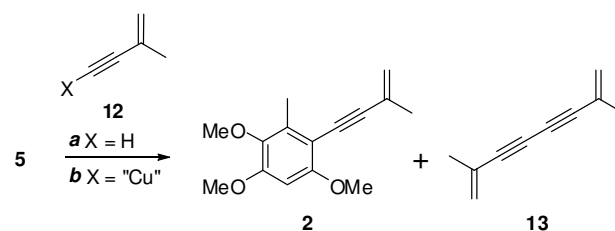
avoided by keeping reaction times short. Methylation of **8** then provided **10**. Treatment with *N*-iodosuccinimide and catalytic trifluoroacetic acid⁸ furnished a quantitative yield of the desired iodide **5**. Alternatively, NaI/Oxone⁹ provided the iodide more cheaply, although in lower yield. The identity of the iodide was confirmed by a 1D NOESY experiment. Irradiation of the aryl proton at 6.41 ppm led to enhancement of two methoxy signals, which cannot occur in the regioisomer **11**.



Scheme 2. Reagents, conditions and yields: (a) POCl₃, DMF, 97%; (b) H₂O₂, H₂SO₄, MeOH, 97% (optimised for **8**); (c) MeI, K₂CO₃, DMF, 96%; (d) NIS, TFA, MeCN, quant.; or I₂, Oxone, H₂O, 77%.

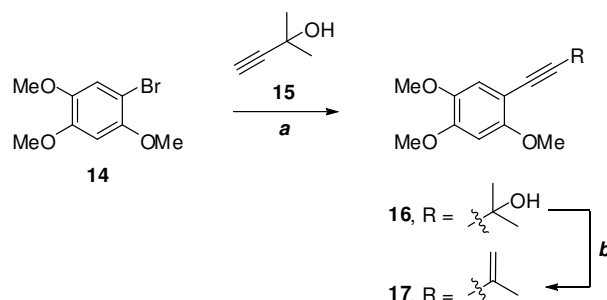
Synthesis of the antrocamphins

Wu and colleagues completed their total synthesis of antrocamphin A with a low-yielding (10%) Sonogashira coupling of iodide **5** with enyne **12** (X = H, Scheme 3).⁵ We also encountered problems with this reaction. Complete conversion to antrocamphin A (**2**) was not achieved despite varying the base, increasing the excess of the terminal alkyne, and carrying out the reaction in a sealed vessel. At best, a conversion of 44% (based on the ¹H NMR spectrum of the crude product) was obtained, with the desired product accompanied by the homocoupled diyne **13** and unreacted iodide **5**. While the volatility of **13** facilitated its simple removal from the crude product, the very similar chromatographic mobility of **5** and antrocamphin A (**2**) made purification virtually impossible. Accordingly we investigated the Castro-Stephens reaction¹⁰ of the copper acetylide **12** (X = "Cu")¹¹ (Scheme 3). Pleasingly, coupling of this species proceeded smoothly in refluxing pyridine, providing antrocamphin A as a yellow solid in 74% yield. The spectroscopic features of synthetic **2** were consistent with the natural product¹ and previously synthesised material.⁵



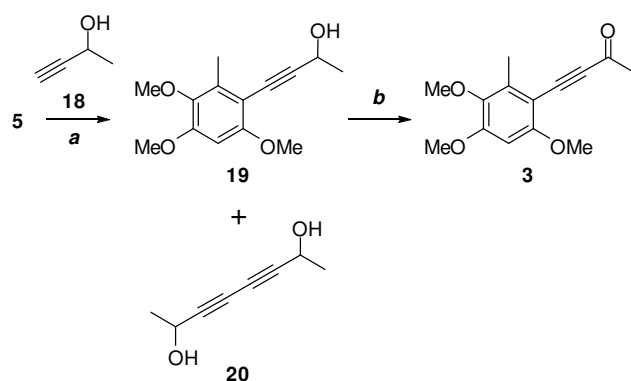
Scheme 3 Reagents, conditions and yields: (a) Pd(PPh₃)₂Cl₂, CuI, NEt₃, MeCN, 80 °C, sealed tube, 44% (as a mixture with **5**); (b) pyridine, reflux, 74% (**2**).

Wu and co-workers have recently applied an efficient, two-step strategy for the installation of the 3-methylbut-3-en-1-ynyl substituent in a very electron-rich substrate **14** in their synthesis of benzocamphorin F **17** (Scheme 4), a co-metabolite of antrocamphin A from *Antrodia camphorata*.¹²



Scheme 4 Wu's synthesis of benzocamphorin F.¹² (a) Pd(PPh₃)₄, CuI, DMF, 85%; (b) MsCl, PhMe, microwave, 92%.

Our attention now turned to antrocamphin B (**3**), which could in principle be derived from the Sonogashira coupling of the iodide **5** with 3-butyne-2-one. However, electron deficient alkynes are poor substrates for the Sonogashira reaction, so we opted for two-step coupling of the propargyl alcohol **18**, followed by oxidation. After some experimentation, the Sonogashira coupling to give **19** was achieved in moderate yield following chromatography and evaporation of the homocoupled diyne **20**, which had similar chromatographic mobility to **19**. Somewhat



Scheme 5 Reagents, conditions and yields: (a) Pd(PPh₃)₂Cl₂, CuI, HNEt₂, DMSO, 75 °C, 58%; (b) MnO₂, DCM, 80%; or, DMSO, Ac₂O, quant.

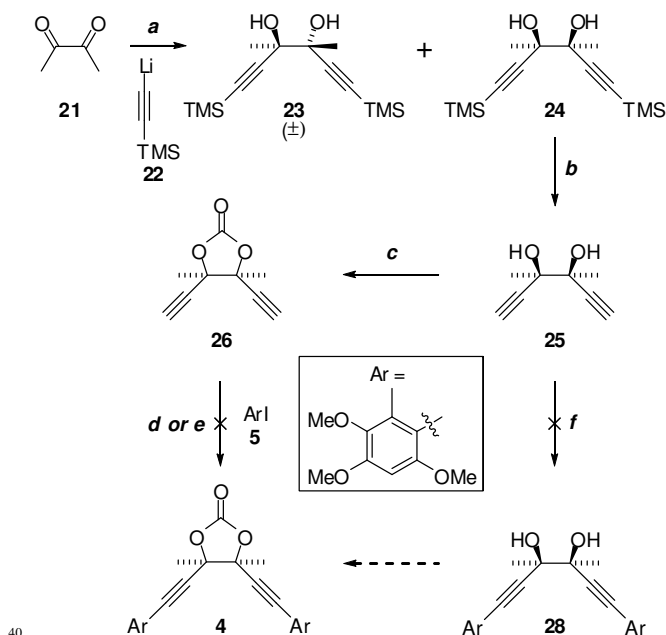
surprisingly, the Castro-Stevens reaction of the copper acetylide derived from **18** failed in this case. Oxidation of **19** with MnO₂¹³ or, more reliably, under modified Swern conditions^{14, 15} provided antrocamphin B (**3**), as a bright yellow solid, in excellent yield. The spectroscopic data derived from **3** were consistent with those reported for the natural product.¹

10

Approaches towards antrodioxolanone

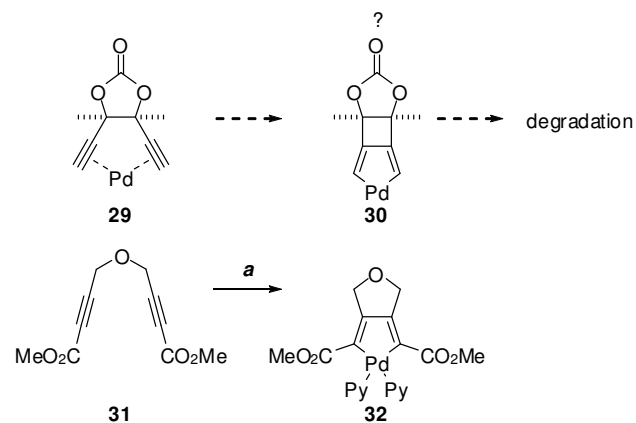
Application of a key double Sonogashira reaction to the synthesis of the more complex antrodioxolane (**4**) required the *meso*-diol **25**, which was prepared in two steps from diacetyl (**21**) and lithium TMS-acetylide (**22**), as described previously (Scheme 6).¹⁶ Cyclocondensation with triphosgene then gave the novel dioxolanone **26**. Unfortunately all attempts to effect the Sonogashira reaction resulted only in the consumption of the diyne **26**, with the iodide **5** recovered essentially quantitatively. In an attempt to emulate the Castro-Stevens reaction that was successful in the synthesis of antrocamphin A, **26** was subjected to the conditions that gave copper acetylide **12** (X = "Cu"). Although the identity of the bright yellow precipitate that formed could not be conclusively assigned, on the assumption that the diacetylide had formed, it was heated with iodide **5**; however, once again, only **5** was recovered.

The failure of the Sonogashira and Castro-Stevens reactions of **26**, and its instability under the reaction conditions, was puzzling. Such reactions of 1,5-diyne are numerous; however, the vast majority of examples involve *o*-ethynylbenzenes. To the best of our knowledge there are no examples with a bridging 5-membered ring. This suggested that the rigid 5-membered-ring, and the *meso* configuration of **26** may predispose the dialkyne to π -chelate palladium (as in **29**), leading to some unknown mode of degradation, perhaps via oxidative cyclisation to the palladacycle **30** (Scheme 7). Although 3-palladabicyclo[3.2.0]hepta-1,4-dienes such as **30** appear to be unprecedented, the [3.3.0]-palladabicyclo **32**, synthesised by oxidative addition to the diene **31**, has been isolated and characterised spectroscopically.¹⁷

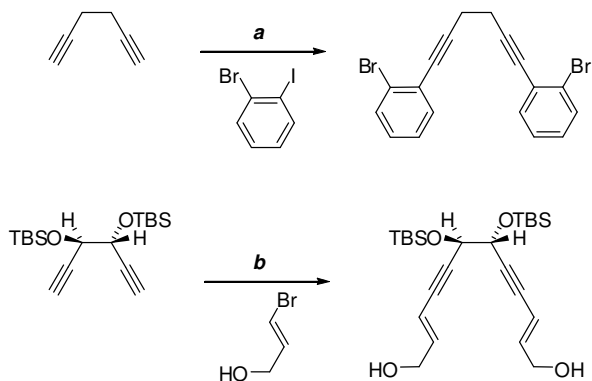


Scheme 6 Reagent, conditions and yields: (a) See reference ¹⁶; (b) K₂CO₃, DCM/MeOH, 96%; (c) CO(OCCl₃)₂, pyridine, THF, 83%; (d) Pd(PPh₃)₂Cl₂; NEt₃ thin film, or with MeCN, or HNEt₂/MeCN, all 0%; (e) 1. HONH₂Cl, CuSO₄·5H₂O, NH₃, H₂O, EtOH (52% based on diacetylide); 2. **5**, pyridine, reflux (0%); (f) Pd(PPh₃)₂Cl₂, NEt₃ or HNEt₂, MeCN, both 0%.

There are just two reported examples of the double Sonogashira reaction of a 1,5-diyne with bridging sp³-hybridised carbon atoms (Scheme 8).^{18, 19} These precedents suggested that, for our purposes, it may be possible to effect the sp-sp² coupling prior to formation of the dioxolane, that is, with diol **25** (Scheme 6), in which greater conformational freedom might disfavour oxidative cyclisation. However, in practice, none of the desired coupling product **28** was isolated.

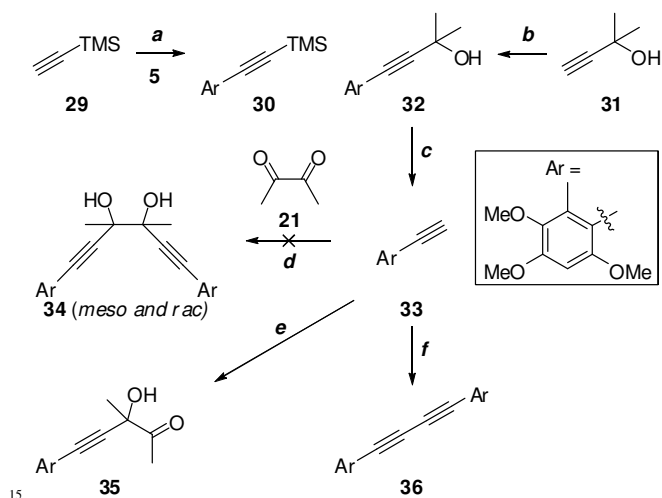


Scheme 7 Reagents, conditions (a) 1. Pd₂(dba)₃·CHCl₃, acetone; 2. pyridine (Py), DCM.¹⁷



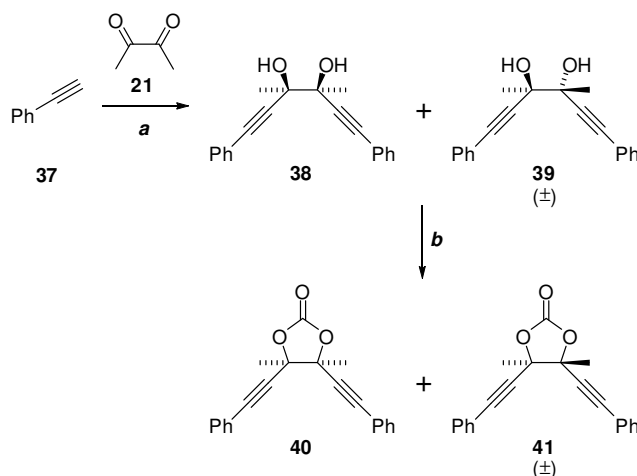
Scheme 8 Reagents, conditions and yields (a) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , NEt_3 , piperidine, 46%;¹⁸ (b). $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , HNEt_2 , 79%.¹⁹

Given the apparent incompatibility of the diynes **25** and **26** with Sonogashira coupling conditions, our attention turned to strategies in which the quaternary stereocentres required for antrodioxolanone are constructed late in the synthesis. The first of these is outlined in Scheme 9. The Sonogashira coupling of TMS-acetylene **29** with iodide **5** suffered from all of the problems associated with the analogous reaction of **12** ($X = \text{H}$, Scheme 3), and as a result the yield of **30** was low. The coupling with the significantly cheaper masked acetylene **31** was much more efficient, and deprotection²⁰ of **32** proceeded smoothly to provide the terminal acetylene **33** in excellent yield.



Scheme 9 Reagents, conditions and yields (a) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , NEt_3 , 21%; (b) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , HNEt_2 , DMSO, 71%; (c) NaOH , PhMe , reflux, 94%; (d) Na , THF, then **21**; or RMgBr , Et_2O and/or THF, then **21** ($R = \text{Et}$ or $i\text{-Pr}$. All gave complex mixtures); (e) EtMgBr , Et_2O , then **21**, 11%; (f) BuLi , THF, then **21**, 17%; or $\text{Pr}_2\text{N}_2\text{OH}$, DMSO, **21**, 10%.

Several attempts at addition of the acetylide, generated in situ by deprotonation of **33** with sodium²¹ or Grignard reagents,²² to diacetyl (**21**), resulted in complex mixtures of products, with none of the glycol **34** detected. On one occasion, the mono-addition product **35** was isolated in low yield. When BuLi ²³ or tetrapropylammonium hydroxide²⁴ were the bases used, the only identifiable product was the diyne **36** arising from oxidative coupling, presumably due to trace contamination by transition metal(s). It is possible that competing deprotonation of diacetyl (**21**) by the acetylide contributes, at least in part, to the failure of

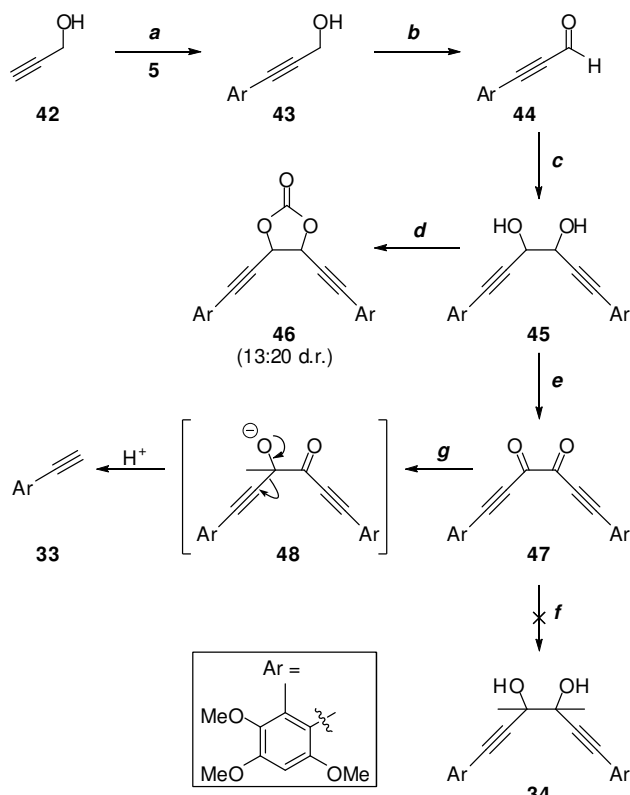


Scheme 10 Reagents, conditions and yields (a) EtMgBr , Et_2O , then **21**, 68% (1:1 mixture of **38** and **39**); (f) $\text{CO}(\text{OCCl}_3)_2$, pyridine, THF, 28% (**40**), 19% (**41**).

these reactions. However, an attempted reaction of the less basic cerium acetylide²⁵ also failed to give any discernible products.

The failure of these reactions, despite the close precedents cited above, including the double addition reaction of lithium TMS-acetylide in our own hands (**21** + **22** \rightarrow **23**, Scheme 6), led us to hypothesise that the electron-rich benzene ring of **33** was somehow negatively impacting the outcome. Indeed, application of the most promising conditions to phenylacetylene (**37**) gave an approximately 1:1 ratio of the diastereomeric diols **38** and **39** in reasonable yield, matching the result reported previously.²² We are unable to explain why the analogous reaction of **33** fails. The cyclocondensation of the diols **38** and **39** gave the corresponding cyclic carbonates **40** and **41**, in low yield, after chromatographic separation.

Given the possible complication of deprotonation of diacetyl by acetylide nucleophiles, we investigated the alternative addition of more reactive methylmetallic nucleophiles to dione **47** (Scheme 11), which lacks appreciably acidic protons. Thus, the Sonogashira reaction of propargyl alcohol (**42**) with iodide **5**, and oxidation of the resultant aryl acetylene **43**, gave aldehyde **44**, which underwent efficient pinacol coupling to give the glycols **45**. It was impossible to determine the diastereomeric ratio from the ^1H NMR spectrum of this mixture due to coincident signals; however, this became apparent upon conversion to the cyclic carbonates **46**. Although it was not possible to distinguish the *cis* (*meso*) from the *trans* (*rac*) isomers, the ^1H NMR spectrum of the mixture did reveal a ~13:20 ratio of diastereomers. This was somewhat immaterial, as the dione **47**, devoid of stereocentres, was the target. Treatment of **45** with MnO_2 led to significant oxidative cleavage, regenerating **44**; this was avoided under modified Swern conditions, providing dione **47** in excellent yield. Unfortunately, the attempted addition reaction of methylmagnesium iodide²⁶ failed entirely, with no evidence for the formation of the desired glycol **34**, or any other identifiable material. With methyllithium,²⁷ terminal acetylene **33** was the only product identified. Presumably this arises from scission of an addition intermediate such as **48**. The instability of such intermediates may partially explain the failure to access the sterically congested glycol moiety required for the synthesis of antrodioxolanone via nucleophilic addition chemistry.

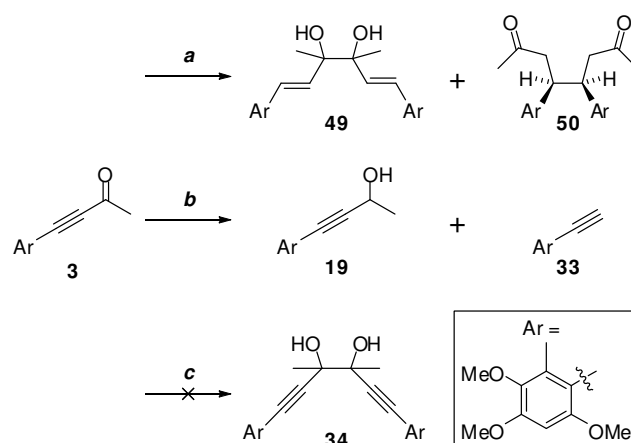


Scheme 11 Reagents, conditions and yields (a) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , HNEt_2 , DMSO , 67%; (b) MnO_2 , DCM , 67% or Ac_2O , DMSO , 55%; (c) Cu/Zn , AcOH , THF , 82%; (d) $\text{CO}(\text{OCCl}_3)_2$, pyridine , DCM , 45%; (e) MnO_2 , DCM , 14% (+ 30% **44**) or Ac_2O , DMSO , 99%; (f) MeMgBr , Et_2O and or THF , 0%; MeLi , $\text{Et}_2\text{O/THF}$, 50% (**33**).

In the original report on the isolation of antrodioxolanone (**4**), it was noted that the natural product may arise biogenetically through an “intermolecular cyclization at the acetyl group” of **3**.¹ Indeed, given the symmetry of antrodioxolanone (**4**), a pinacol coupling of antrocamphin B (**3**) seemed a plausible biosynthetic step, and an appealing means to construct the contiguous quaternary stereocentres in a total synthesis. This realisation led us to explore the pinacol coupling of the ynal **44** described above (Scheme 11). In parallel, we also investigated pinacol couplings of antrocamphin B.

We are unaware of any biosynthetic examples of pinacol couplings. However, photochemically-induced pinacol coupling of an aryl alkynyl ketone has been observed upon irradiation at 300 nm.²⁸ It is conceivable that a non-enzymatic, sunlight-induced pinacol coupling of antrocamphin B (**3**) might be involved in the biosynthesis of antrodioxolanone (**4**). This led us to irradiate solutions of **3** (Scheme 12). However, in all cases no reaction was detected by TLC.

More conventional metal-mediated pinacol couplings were then investigated. The Cu/Zn couple that worked well for ynal **44** (Scheme 11) did indeed give a pinacol coupling product with antrocamphin B (**3**), but unfortunately accompanied by semi-reduction of the alkynes to give a *trans*-diene **49**. Although this appeared to be a single diastereomer, it was not possible to define the relative configuration with the spectroscopic data available. In addition, the dihydrostilbene **50**, arising from reductive coupling at the benzylic position, was isolated in low yield. X-ray



Scheme 12 Reagents, conditions and yields (a) Cu/Zn , AcOH , THF , 22% (**49**), 8% (**50**); (b) Na , PhBr , PhMe , 23% (**19**), 4% (**33**); (c) NEt_3 , UV (TLC lamp) or *i*- PrOH , AcOH , ambient lab light then direct sunlight.

crystallography revealed this to be the *meso* isomer (Fig 2), although the formation of the chiral diastereomers cannot be ruled out, as not all products of this reaction were able to be purified. The attempted sodium/bromobenzene-promoted pinacol coupling²⁹ of **3** resulted in degradation, whereas no reaction was observed with this reductant system in carbon tetrachloride or cyclohexane solutions. In toluene, the secondary alcohol **19**, and the terminal alkyne **33**, presumably resulting from scission of the alkoxide precursor to **19**, were the only detectable products. Treatment of **3** with TiCl_4 -TBAI, which is an effective promoter of pinacol coupling for aryl methyl ketones,³⁰ gave a complex mixture of products.

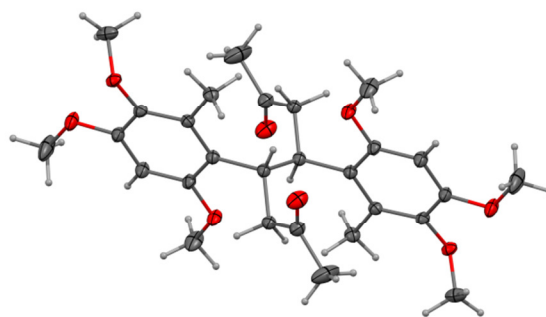


Fig. 2 Representation of the crystal structure of **50**. Ellipsoids are shown at 50% probability amplitudes with hydrogen atoms assigned arbitrary radii.

Of the reducing agents investigated, only SmI_2 ^{14,15} provided the pinacol coupling products **34a** and **34b**, in 55% yield, but unfortunately favouring the chiral isomers **34b** 10:1. The diastereomers were separable by HPLC but, disappointingly, and hampered by material availability, attempts to convert the *meso* isomer into antrodioxolanone were unsuccessful. When the 10:1 mixture of diastereomeric glycols was treated with triphosgene, only the chiral *trans*-isomer, (\pm)-*epi*-antrodioxolanone (**51**) was isolated in very low yield, as confirmed with an X-ray crystal structure (Fig 3). The yield of **51** could neither be improved using NEt_3 or DMAP as catalysts, nor carbonyldiimidazole as electrophile, and presumably results from steric congestion in the

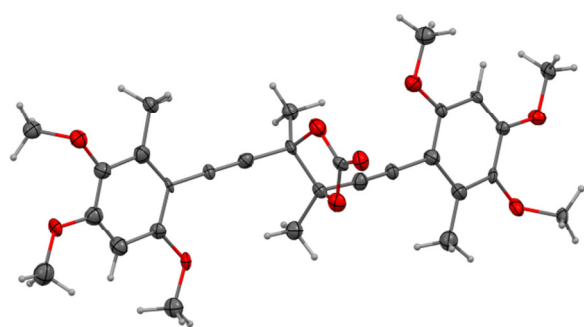
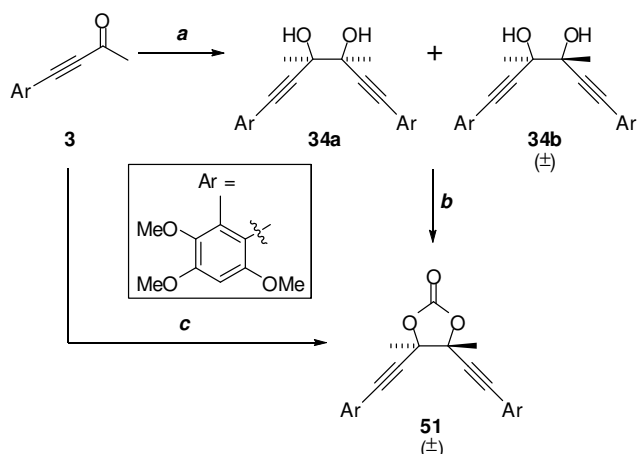


Fig. 3 Representation of the crystal structure of (±)-epi-antrodioxolanone (**51**). *R,R*-enantiomer shown. Ellipsoids are shown at 50% probability amplitudes with hydrogen atoms assigned arbitrary radii.

bis-tertiary glycol. A final attempt at the one-pot pinacol coupling/cyclisation using SmI_2 and methyl chloroformate³¹ gave **51** directly, albeit in low yield, with none of the *meso* natural product **4** detected.



Scheme 13 Reagents, conditions and yields (a) SmI_2 , THF, 55% (1:10 mixture of **34a**:**34b**); (b) $\text{CO}(\text{OCCl}_3)_2$, pyridine, DCM, 16% (c) 1. SmI_2 , THF; 2. ClCO_2Me (32%).

Anti-inflammatory activity

Following their initial isolation and structure elucidations, the antrocamphins and antrodioxolanone were assessed for anti-inflammatory effects through their impact on superoxide anion production by neutrophils, induced by the inflammatory cytokine fMLP (*N*-formyl-Met-Leu-Phe).¹ Antrocamphin B and antrodioxolanone showed no activity in this assay, but antrocamphin A suppressed superoxide production with an IC_{50} of $9 \pm 3 \mu\text{M}$, more effectively than ibuprofen ($\text{IC}_{50} = 28 \pm 3 \mu\text{M}$).¹ Synthetic antrocamphin was later also shown to inhibit the fMLP-induced excretion of elastase by human neutrophils, and many analogues of the natural product more potently inhibited superoxide generation by these cells.⁵

Additional mode of action studies on antrocamphin A were conducted by Wang and coworkers.³ The natural product dose-dependently suppressed the production of inflammatory cytokines NO and prostaglandin E_2 in lipopolysaccharide-challenged macrophages (RAW 264.7 cells). The expression of

inflammatory enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were also downregulated by antrocamphin A. The authors hypothesised that this could be due to suppression of NF κ B, a transcription factor that is a central player in the inflammatory cascade. Cytosolic NF κ B is bound by the inhibitor I κ B. Phosphorylation of I κ B by the kinase IKK causes the NF κ B–I κ B complex to dissociate, allowing NF κ B to enter the nucleus, where it induces transcription of a host of genes involved in the inflammatory response. Nuclear accumulation of NF κ B was indeed dose-dependently decreased by antrocamphin A. Concurrently, expression of I κ B increased, while that of the phosphorylated form of IKK, decreased.³

We have recently developed a cellular assay to determine the effects of novel thalidomide derivatives on the NF κ B activation pathway, as a measure of anti-inflammatory activity.^{32–34} To measure inhibition of NF κ B pathway signalling, a Tumour Necrosis Factor (TNF) transcriptional reporter cell line was constructed by linking the green fluorescent protein (GFP) reporter gene to the NF κ B-responsive human TNF promoter. The construct was then inserted into the genome of the human T cell line, Jurkat E6-1 to generate the reporter line, FRT-Jurkat TNF, as previously described.^{35, 36} As a measure of TNF promoter activity, GFP activity can be quantitated by flow cytometry. This method has the added advantage of being able to concurrently assess the cytotoxicity of each compound, by comparing forward- and side-scatter of light during flow cytometry.

In the current study, antrocamphin A dose-dependently reduced the amount of expression by the TNF reporter line (Figure 4). The results suggest an IC_{50} for NF κ B-induced expression inhibition of approximately $100 \mu\text{M}$; however, it was not possible to determine this value accurately as, at the higher concentrations, there was a significant effect on cell viability (Fig. 4). Although the issue seems to have been avoided in earlier publications, antrocamphin A does exhibit dose-dependent cytotoxicity towards RAW 264.7 cells, causing approximately 30% cell death at $20 \mu\text{g}\cdot\text{mL}^{-1}$.³ More recently, antrocamphin A was shown to be toxic to four human tumour-derived cell lines – Doay (breast medulloblastoma), Hep2 (laryngeal carcinoma), MCF-7 (breast adenocarcinoma) and Hela (cervical epitheloid carcinoma) – with ED_{50} values $\leq 10 \mu\text{g}\cdot\text{mL}^{-1}$.⁴

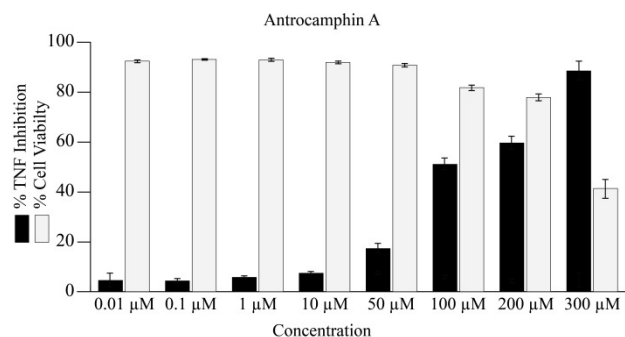


Fig. 4 Inhibition of TNF-reporter gene expression and consequence on cell viability following treatment with antrocamphin A (**2**) for 24 h. Data represent geometric mean expression levels of GFP driven by a TNF promoter, measured by flow cytometry ($n = 3$, bars represent mean \pm SEM). Cell viability was assessed by comparing forward- and side-scatter as a measure of cellular size and granularity. The cell population in each sample that exhibited low granularity were considered dead, as confirmed by propidium iodide staining.

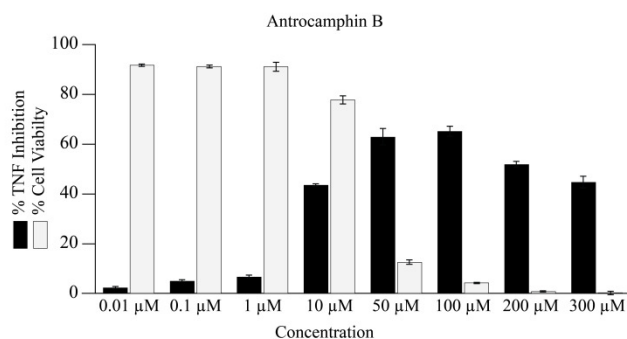


Fig. 5 Inhibition of TNF promoter transcriptional activity and consequence on cell viability following treatment with antrocamphin B (3) for 24 h. Data represent geometric mean expression levels of GFP driven by the TNF promoter measured by flow cytometry ($n = 3$, bars represent mean \pm SEM). Cell viability was assessed as described in the figure 4 caption.

It seems that the biological activity of antrocamphin B (3) has not been considered since its isolation. We also assessed the ability of this compound to suppress NF κ B TNF-promoter mediated transcription. Although there is evidence for the inhibition of TNF transcription, there is no clear dose-response relationship and the data are clearly complicated by the cytotoxicity of the compound (Figure 5, $IC_{50} = 10.7 \pm 0.3 \mu\text{M}$ [std. dev]). This is not surprising given that antrocamphin B is a Michael acceptor.

Some synthetic intermediates and analogues of the antrocamphins and antrodioxolanone were also briefly assessed in the TNF inhibition assay (Figure 6). Interestingly, **40** the analogue of antrodioxolanone (4) possessing phenyl substituents in place of the oxygenated aromatic substituents in the natural product, did inhibit TNF-induced expression at 100 μM with little effect on cell viability. The mixture of its *trans*-diastereomers **41** on the other hand, was quite cytotoxic, killing approximately 60% of cells at 10 μM . The ynal **44** also inhibited TNF expression, but with accompanying cell death. None of the compounds displayed activity warranting more rigorous examination

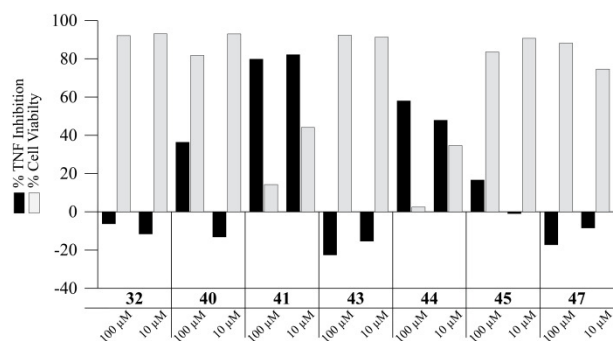


Fig. 6 Inhibition of TNF reporter gene expression and consequence on cell viability following treatment with synthetic intermediates and analogues of the antrocamphins and antrodioxolanone for 24 h. Data represent geometric mean expression levels of GFP driven by a TNF promoter measured by flow cytometry.

Conclusions

An improved synthesis of the anti-inflammatory natural product antrocamphin A (2), involving a key Castro-Stephens reaction, has been devised, along with the first synthesis of its congener antrocamphin B (3). Several approaches to the synthesis of the more complex antrodioxolanone (4) were thwarted, including a route involving a possibly biomimetic pinacol coupling of antrocamphin B. This latter strategy did, however, provide racemic *epi*-antrodioxolanone (51). The sterically congested 4,5-diethynyldioxolanone core of antrodioxolanone is unique amongst natural products and its stereoselective synthesis, in the presence of electron rich pendant aromatic rings, presents quite a challenge.

Antrocamphin A (2) was shown to inhibit TNF expression with modest potency, supporting an earlier hypothesis³ that its anti-inflammatory effects arise, at least in part, by interfering with the nuclear localisation of the transcription factor NF κ B. The potency of action determined herein is approximately an order of magnitude less than downstream measures of anti-inflammatory activity reported previously, which is not surprising for a drug acting on a signalling pathway that regulates the expression of genes associated with the inflammatory response.

Despite being a constituent of a Chinese traditional medicine that has presumably been used without severe adverse effects for some time, there is mounting evidence that antrocamphin A is toxic to some cell types. Whether this cytotoxicity is linked with its interference in the inflammatory signalling cascade and/or is selective towards cancerous cell lines, and whether antrocamphin A and related compounds exhibit safe therapeutic indices, remains to be established.

Experimental

General details

General details are as described previously.³⁷

Crystallography

Crystallographic data for **50** and **51** were collected at 100(2) K on an Oxford Diffraction Gemini or Xcalibur diffractometer fitted with Mo K α radiation. Following multi-scan absorption corrections and solution by direct methods, the structures were refined against F^2 with full-matrix least-squares using the program SHELXL-97.³⁸ All H-atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atoms. Anisotropic displacement parameters were employed for the non-hydrogen atoms.

2,4-Dimethoxy-6-methylbenzaldehyde (7)⁶

POCl_3 (14.5 mL, 0.156 mol) was added dropwise to a solution of 3,5-dimethoxytoluene (19 g, 0.12 mol) in DMF (100 mL) at 0°C. The reaction was allowed to warm to room temperature over 24 h before being poured slowly into cold H_2O (100 mL). After 15 min the suspension was further diluted with H_2O (300 mL) and saturated NaHCO_3 (100 mL), stirred overnight, then extracted with EtOAc (4×50 mL). The extract was dried and evaporated to give **7** as a white powder (21.9 g, 97%) sufficiently pure for the

next step, m.p. = 64–65 °C [lit.⁶ 64–65 °C]. ¹H NMR (400 MHz) δ 10.48 (s, 1H, CHO), 6.32 (s, 2H, 2 \times ArH), 3.87 (s, 3H, OMe), 3.85 (s, 3H, OMe), 2.58 (s, 3H, Me). The ¹H NMR data are similar to those acquired at 300 MHz previously reported.³⁹

2,4-Dimethoxy-6-methylphenol (**8**)

30% Aqueous H₂O₂ (15.2 mL, 149 mmol) was added dropwise to a stirred solution of **7** (21.9 g, 122 mmol) and concentrated H₂SO₄ (0.25 mL, 4.7 mmol) in MeOH (100 mL) at 0 °C. After 20 min the precipitate that had formed was filtered, washed with H₂O (3 \times 20 mL) and dried under vacuum to give **8** a white solid (19.8 g, 97%), m.p. = 103–104 °C [lit.⁴⁰ 103–104 °C]. ¹H NMR (400 MHz) δ 6.35 (d, *J* = 2.8 Hz, 1H, ArH), 6.29 (d, *J* = 2.8 Hz, 1H, ArH), 5.26 (s, 1H, OH), 3.85 (s, 3H, OMe), 3.75 (s, 3H, OMe), 2.24 (s, 3H, Me). The ¹H NMR data are similar to those acquired at 60 MHz previously reported.⁴⁰

2,3,5-Trimethoxytoluene (**10**)⁴¹

MeI (1.36 mL, 22.8 mmol) was added to a stirred suspension of **8** (2.90 g, 17.2 mmol) and K₂CO₃ (4.7 g, 34 mmol) in dry DMF (30 mL) under argon. The reaction mixture was stirred in the dark for 24 h then quenched with ice-cold 1 M HCl (200 mL). The aqueous phase was extracted with ether (6 \times 80 mL). The extract was washed with saturated NaHCO₃ (50 mL), saturated NH₄Cl (50 mL) and water (50 mL), then dried and evaporated to give **10** as a colourless oil (2.96 g, 96%). ¹H NMR (500 MHz) δ 6.35 (1H, d, *J* = 3.0 Hz, H6), 6.28 (1H, d, *J* = 3.0 Hz, H4), 3.83 (3H, s, MeO), 3.76 (3H, s, MeO), 3.74 (3H, s, MeO), 2.25 (3H, s, Me). The ¹H NMR data are similar to those acquired at 80 MHz previously reported.⁴¹

2,3,5-Trimethoxy-6-iodotoluene (**5**)

Method 1: *N*-iodosuccinimide (3.70 g, 16.6 mmol) was added to a stirred solution of **10** (2.70 g, 14.8 mmol) and trifluoroacetic acid (350 μ L, 4.5 mmol) in dry MeCN (60 mL) under argon. The reaction mixture was stirred in the dark for 30 min then poured into ice-water (300 mL) and extracted with DCM (4 \times 60 mL). The extract was washed with H₂O (50 mL), dried and evaporated to give **5** as a yellow solid (4.60 g, quant.), which crystallised from MeOH as white needles, m.p. = 90–93 °C. *R*_f (10% EtOAc/hexanes) 0.4. ¹H NMR (500 MHz): δ 6.41 (1H, s, H6), 3.88 (3H, s, 5-MeO), 3.86 (3H, s, 1-MeO), 3.72 (3H, s, 4-MeO), 2.43 (3H, s, Me). ¹³C NMR (125 MHz): δ 154.9 (ArO), 153.4 (ArO), 141.6 (ArO), 136.4 (Ar-Me), 95.0 (Ar-H), 82.3 (C1), 60.8 (MeO), 57.0 (MeO), 56.1 (MeO), 21.8 (Me). MS (EI) *m/z* 308 (M, 100%), 293 (79), 265 (30), 250 (13); HRMS observed: 307.9910 C₁₀H₁₃IO₃⁺ requires: 307.9909. Microanalysis found: C 39.1, H 4.1%; calculated for C₁₀H₁₃IO₃: C 39.0, H 4.3%. The ¹H NMR data are identical to those acquired at 200 MHz and reported previously.⁵

Method 2: A mixture of Oxone (0.28 g, 0.46 mmol), **10** (0.16 g, 0.89 mmol) and NaI (0.14 g, 0.91 mmol) in H₂O (7 mL) was heated under reflux for 4 h, then cooled, diluted with H₂O (30 mL) and extracted with EtOAc (3 \times 10 mL). The extract was washed with 10% Na₂S₂O₄ (2 \times 10 mL), dried and evaporated to give a yellow solid, which crystallised from MeOH to give **10** as pale-yellow needles (0.21 g, 77%), spectroscopically identical

with the material described above.

1,2,5-Trimethoxy-3-methyl-4-(3-methylbut-3-en-1-yn-1-yl)benzene, antrocamphin A (**2**)

Copper isopropenylacetylide (**12b**)⁴² (77 mg, 0.60 mmol) was added to a stirred solution of **5** (62 mg, 0.20 mmol) in anhydrous pyridine (1.5 mL) under argon, and the reaction mixture was heated under reflux for 24 h. After cooling, the reaction mixture was filtered, and the filtrate was diluted with H₂O (50 mL) and extracted with ether (4 \times 30 mL). The ether extract was evaporated to give a yellow oil, which was subjected to RSF. Elution with EtOAc:hexanes 1:19 gave **2** as a yellow solid (36 mg, 74%), which crystallised from hexanes as a yellow powder, m.p. = 39–41 °C [lit.^{1, 5} oil]. *R*_f (20% EtOAc:hexanes) 0.5; IR ν_{max} cm⁻¹: 2197 (C \equiv C). ¹H NMR (500 MHz, CDCl₃) δ 6.33 (s, 1H, H6'), 5.37 (m, 1H, H4), 5.25 (m, 1H, H4), 3.88 (s, 3H, MeO), 3.87 (s, 3H, MeO), 3.72 (s, 3H, MeO), 2.36 (s, 3H, 3'-Me), 2.01 (t, *J*_{3-Me,4} = 1 Hz, 3H, 3-Me); ¹³C NMR (125.8 MHz; CDCl₃) δ 157.4 (ArO), 153.6 (ArO), 141.3 (ArO), 135.5 (3'-Me), 127.5 (C3), 120.9 (C4), 105.1 (C4'), 97.7 (C2), 94.6 (C6'), 83.7 (C1), 60.6 (MeO), 56.5 (MeO), 56.0 (MeO), 23.9 (3-Me), 14.2 (3'-Me); MS (EI) *m/z* 248 (63%), 246 (M, 100), 233 (53), 231 (64); HRMS observed: 246.1257 C₁₅H₁₈O₃⁺ requires: 246.1256. The spectroscopic data match those reported previously.^{1, 5}

4-(3,4,6-Trimethoxy-2-methylphenyl)but-3-yn-2-ol (**19**)

A Young's flask was charged with **5** (2.62 g, 8.51 mmol), CuI (71 mg, 5 mol%), Pd(PPh₃)₂Cl₂ (77 mg, 1.5 mol%), DMSO (20 mL) and Et₂NH (4 mL, 0.04 mol), then briefly evacuated and back filled with argon. But-3-yn-2-ol (**18**) (1.0 mL, 13 mmol) was added and the flask was sealed [CAUTION: safety shield]. The mixture was stirred at 65 °C for 16 h, then cooled to room temperature, and diluted with H₂O (100 mL) and 1M HCl (20 mL). The aqueous solution was extracted with EtOAc (3 \times 30 mL), dried and evaporated to give a brown oil, which was subjected to flash chromatography. Elution with 40% EtOAc/hexanes gave **19** (1.24 g, 58%) as a pale yellow solid, which crystallised from MeOH as pale yellow needles, m.p. = 119–124 °C. *R*_f (20% EtOAc:hexanes) 0.1; IR ν_{max} cm⁻¹: 3600–3100 (OH), 2219 (C \equiv C); ¹H NMR (500 MHz, CDCl₃) δ 6.32 (s, 1H, H5'), 4.83 (m, 1H, H2), 3.88 (s, 3H, MeO), 3.86 (s, 3H, MeO), 3.71 (s, 3H, MeO), 2.34 (s, 3H, 2'-Me), 2.06 (br d, *J*_{OH,2} = 5 Hz, 1H, OH), 1.57 (d, *J*_{1,2} = 7 Hz, 3H, 1-Me); ¹³C NMR (125.8 MHz; CDCl₃) δ 157.5 (ArO), 153.7 (ArO), 141.3 (ArO), 135.7 (C2'), 104.2 (C1'), 98.1 (C3 or 4), 94.4 (C5'), 79.1 (C3 or 4), 60.6 (MeO), 59.3 (C2), 56.4 (MeO), 56.0 (MeO), 24.8 (2'-Me), 14.2 (C1); MS (EI) *m/z* 250 (M, 36%) 232 (85), 86 (63), 84 (100); HRMS observed: 250.1202 C₁₄H₁₈O₄⁺ requires: 250.1205; Microanalysis found: C 66.4, H 7.1%; calculated for C₁₄H₁₈O₄: C 67.2, H 7.2%.

4-(3,4,6-Trimethoxy-2-methylphenyl)but-3-yn-2-one, antrocamphin B (**3**)

Method 1: Activated MnO₂ (420 mg, 4.8 mmol) was added to a stirred solution of **19** (60 mg, 0.24 mmol) in anhydrous DCM (2 mL) under argon. The reaction mixture was stirred for 24 h then vacuum filtered through a Celite plug and washed through with DCM. Evaporation of the filtrate gave **5** as a yellow solid (47 mg,

80%), which crystallised from MeOH as bright yellow needles, m.p. = 101–108 °C. R_f (20% EtOAc:hexanes) 0.15; IR ν_{\max} cm^{-1} : 2180 (C≡C), 1646 (C=O). ^1H NMR (500 MHz, CDCl_3) δ 6.32 (s, 1H, H^{5'}), 3.91 (s, 3H, MeO), 3.89 (s, 3H, MeO), 3.73 (s, 3H, MeO), 2.46 (s, 3H, 1-Me), 2.39 (s, 3H, 2'-Me); ^{13}C NMR (125.8 MHz; CDCl_3) δ 184.7 (C=O), 159.9 (ArO), 156.3 (ArO), 141.3 (ArO), 137.4 (C^{2'}), 101.4 (C^{1'}), 96.4 (C3), 94.0 (C5'), 88.0 (C4), 60.6 (MeO), 56.3 (MeO), 56.0 (MeO), 32.9 (1-Me), 14.2 (2'-Me); MS (EI) m/z 248 (M, 100%) 233 (85), 205 (13); HRMS observed: 248.1044, $\text{C}_{14}\text{H}_{16}\text{O}_4$ requires: 248.1049. The spectroscopic data matched those reported.¹

Method 2: Ac_2O (10 mL, 0.11 mol) was added to a stirred solution of **19** (1.24 g, 4.95 mmol) in DMSO (40 mL). After 24 h the reaction mixture was diluted with H_2O (100 mL) and the resultant precipitate was collected by vacuum filtration. The filtrate was extracted with EtOAc (3 × 30 mL). The extract was dried, combined with the precipitate, and evaporated to give **3** as a yellow solid (1.21 g, quant.), identical with the material described above.

meso-3,4-Dimethyl-hexa-1,5-diyne-3,4-diol (**25**)

K_2CO_3 (155 mg, 1.13 mmol) was added to a stirred solution of *meso*-3,4-dimethyl-1,6-bis(trimethylsilyl)hexa-1,5-diyne-3,4-diol (**24**)⁴³ (210 mg, 0.75 mmol) in MeOH/DCM (1:1, 3 mL) under argon. The resulting slurry was stirred for 3 h then vacuum filtered through Celite and rinsed through with DCM. The filtrate was evaporated to give a colourless oil, which was subjected to RSF. Elution with 20% EtOAc:hexanes gave **25** as a white solid (97 mg, 94%). ^1H NMR (400 MHz, CDCl_3) δ 2.80 (s, 2H, OH), 2.54 (s, 2H, CH), 1.55 (s, 6H, Me). The ^1H NMR spectrum matched the data reported.⁴³

meso-4,5-Diethynyl-4,5-dimethyl-1,3-dioxolan-2-one (**26**)

A solution of triphosgene (60 mg, 0.2 mmol) in DCM (0.5 mL) was added dropwise to a stirred solution of **25** (35 mg, 0.25 mmol) and pyridine (99 mg, 1.25 mmol) in anhydrous DCM (0.5 mL) at -78 °C under argon. The reaction mixture was warmed to 0 °C slowly (over 3 h) and stirred at 0 °C for 1 h, then quenched with saturated NH_4Cl (20 mL) and extracted with DCM (4 × 20 mL). The extract was washed with 1 M HCl (20 mL), brine (20 mL), saturated NaHCO_3 (20 mL) and brine (20 mL), dried and evaporated to give **26** as a white solid (35 mg, 83%), which crystallised from hexanes/EtOAc as a white powder, m.p. = 59–62 °C. R_f (20% EtOAc:hexanes) 0.28; IR ν_{\max} cm^{-1} : 3287 ($\equiv\text{CH}$), 2133 (C≡C), 1798 (C=O); ^1H NMR (500 MHz, CDCl_3) δ 2.83 (s, 2H, 2 × CH), 1.71 (s, 6H, 2 × Me); ^{13}C NMR (125.8 MHz; CDCl_3) δ 151.8 (CO), 81.7 (C4/5-C alkyne), 78.7 (CH), 78.6 (C4/5), 22.3 (Me); MS (CI) m/z 165 [M]⁺⁺ (100), 103 (10); HRMS observed: 165.0551, $\text{C}_9\text{H}_9\text{O}_3$ ⁺⁺ requires: 165.0552.

1,2,5-Trimethoxy-3-methyl-4-(trimethylsilylethynyl)benzene (**30**)

A stirred solution of **5** (308 mg, 1.11 mmol) in triethylamine (1.25 mL) was evacuated and back filled with argon (× 3) then treated with trimethylsilylacetylene (150 μL , 1.1 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (7 mg, 1 mol%) and CuI (9 mg, 5 mol%). The reaction vessel was sealed [CAUTION: safety shield] then stirred

at 60 °C for 72 h. ^1H NMR analysis of an aliquot after this time showed the starting material was only 16% consumed. Additional equivalents of catalysts and trimethylsilylacetylene (amounts as above) were added and the reaction mixture heated at 60 °C in a sealed tube for another 72 h. The process was repeated with fresh equivalents of catalyst and alkyne (amounts as above) for another 96 h then the reaction mixture was diluted with H_2O (50 mL) and extracted with ether (4 × 40 mL). The extract was dried and evaporated to give an orange oil, which was subjected to RSF. Elution with 10% EtOAc:hexanes gave **30** as a white solid (59 mg, 21%), which crystallised from hexanes/EtOAc as a white powder, m.p. = 56–59 °C. R_f (10% EtOAc:hexanes) 0.3; IR ν_{\max} cm^{-1} : 2147 (C≡C); ^1H NMR (500 MHz, CDCl_3) δ 6.30 (s, 1H, H^{6'}), 3.87 (s, 3H, MeO), 3.86 (s, 3H, MeO), 3.71 (s, 3H, MeO), 2.35 (s, 3H, Me), 0.26 (s, 9H, Si(Me)₃); ^{13}C NMR (125.8 MHz; CDCl_3) δ 157.9 (ArO), 153.8 (ArO), 141.3 (ArO), 136.1 (3'-Me), 105.0 (C^{4'} or 1 or 2), 101.3 (C^{4'} or 1 or 2), 100.3 (C^{4'} or 1 or 2), 94.5 (C^{6'}), 60.6 (MeO), 56.5 (MeO), 55.9 (MeO), 14.2 (3'-Me), 0.41 (Si(Me)₃); MS (EI) m/z 278 [M]⁺⁺, (100%), 263 (83), 248 (23), 233 (24); HRMS observed: 278.1345, $\text{C}_{15}\text{H}_{22}\text{O}_3\text{Si}$ requires: 278.1338

2-Methyl-4-(3,4,6-trimethoxy-2-methylphenyl)but-3-yne-2-ol (**32**)

A stirred mixture of **5** (3.23 g, 10.5 mmol), CuI (64 mg, 3.4 mol%), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (89 mg, 1.3 mol%) and DMSO (30 mL) in a Young's flask was evacuated and backfilled with argon (× 3). Et_2NH (5 mL, 0.05 mol) and 2-methylbut-3-yn-2-ol (**31**) (3 mL, 0.03 mole) were added and the vessel was sealed and stirred at 70 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with H_2O (150 mL) and 1M HCl (30 mL), and extracted with EtOAc (4 × 30 mL). The extract was washed with brine (30 mL), dried and evaporated to give a brown oil, which was subjected to flash chromatography. Elution with 40% EtOAc:hexanes yielded **32** (1.98 g, 71%) as a white solid, m.p. = 94–96 °C. R_f (40% EtOAc:hexanes): 0.2; IR (KBr) ν_{\max} cm^{-1} : 3600–3000 (OH), 2219 (C≡C). ^1H NMR (400 MHz): δ 6.42 (1H, s, H^{5'}), 3.87 (3H, s, OMe), 3.85 (3H, s, OMe), 3.71 (3H, s, OMe), 2.33 (3H, s, C^{2'}-Me), 2.13 (1H, s, OH), 1.64 (6H, s, C^{2'}-Me). ^{13}C NMR (100 MHz) δ 157.6 (C^{4'} or C^{6'}), 153.7 (C^{4'} or C^{6'}), 141.4 (C^{3'}), 135.7 (C^{2'}), 104.6 (C^{1'}), 101.1 (C³ & C⁴), 94.8 (C^{5'}); 66.2 (C²), 60.7 (OMe), 56.6 (OMe), 56.1 (OMe), 32.0 (C¹ & C²-Me), 14.3 (C^{2'}-Me). MS (EI) m/z : 264.1 [M]⁺⁺ (18), 249.1 [$\text{M}-\text{Me}$]⁺ (15), 246.1 [$\text{M}-\text{H}_2\text{O}$]⁺⁺ (100). HRMS (EI): observed, 264.1363. $\text{C}_{15}\text{H}_{20}\text{O}_4$ ⁺⁺ requires 264.1362.

2-Ethynyl-1,4,5-trimethoxy-3-methylbenzene (**33**)

Crushed, dry NaOH (0.18 g, 4.4 mmol) was added to a stirred solution of **32** (0.69 g, 2.6 mmol) in toluene (12 mL) and the mixture was heated under reflux. After 6 h the mixture was cooled to room temperature, diluted with H_2O (50 mL) and 1M HCl (10 mL) and extracted with EtOAc (3 × 10 mL). The extract was washed with brine (30 mL), dried and evaporated to give a brown solid, which was subjected to flash chromatography. Elution with 10% EtOAc:hexanes yielded **32** as a white solid (0.51 g, 94%), m.p. = 86–90 °C. R_f (20% EtOAc:hexanes): 0.2; IR (KBr) ν_{\max} cm^{-1} : 3284 ($\equiv\text{C}-\text{H}$), 2150 (C≡C). ^1H NMR (400 MHz): δ 6.34 (s, 1H, H⁵), 3.88 (s, 6H, 2 × OMe), 3.72 (s, 3H,

OMe), 3.45 (s, 1H, $\equiv\text{CH}$), 2.37 (3H, s, Me). ^{13}C NMR (100 MHz) δ 158.1 (C4 or C6), 159.7 (C4 or C6), 141.0 (C3), 136.0 (C2), 103.5 (C1), 94.1 (C5), 83.6 ($\equiv\text{CH}$); 78.8 (ArC \equiv), 60.4 (OMe), 56.2 (OMe), 55.8 (OMe), 14.0 (Me). MS (EI) m/z : 206 [M] $^{+}$ (100%), 191 [M-Me] $^{+}$ (99). HRMS (EI) observed: 206.0948, $\text{C}_{10}\text{H}_9\text{O}_3$ $^{+}$ requires: 206.0943.

3-Hydroxy-3-methyl-5-(3,4,6-trimethoxy-2-methylphenyl)pent-4-yn-2-one (35)

A 0.20 M solution of EtMgBr (5.0 mL, 1.0 mmol) in Et $_2$ O was added dropwise to a stirred solution of **33** (0.21 g, 1.0 mmol) in Et $_2$ O (5 mL) under argon. The reaction mixture was heated under reflux for 2.5 h, then cooled to room temperature and treated dropwise with a solution of 2,3-butanedione (0.18 mL, 2.1 mmol) in Et $_2$ O (2 mL). The reaction mixture was heated for 24 h under reflux, then cooled, diluted with H $_2$ O (50 mL) and extracted with EtOAc (3 \times 20 mL). The extract was washed with brine (30 mL), dried and evaporated to give a yellow oil, which was subjected to flash chromatography. Elution with 10% EtOAc/hexanes yielded **35** as a white solid (32 mg, 11%), m.p. = 86–88 $^{\circ}\text{C}$. R $_f$ (40% EtOAc/hexanes): 0.25; IR (thin film) ν_{max} cm $^{-1}$: 3100–3700 (OH), 2218 (C \equiv C), 1720 (C=O). ^1H NMR (400 MHz): δ 6.30 (s, 1H, H5'), 4.12 (s, 1H, OH), 3.87 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.70 (s, 3H, OMe), 2.49 (s, 3H, C2'-Me), 2.32 (s, 3H, H1), 1.72 (s, 3H, C3-Me). ^{13}C NMR (100 MHz) δ 206.2 (C2), 157.9 (C4' or C6'), 154.0 (C4' or C6'), 141.1 (C3'), 135.1 (C2'), 102.6 (C1'), 94.8 (C5), 94.3 (C5'), 81.1 (C4), 73.2 (C3), 60.5 (OMe), 56.2 (OMe), 55.8 (OMe), 27.3 (C1), 23.4 (C3-Me), 14.1 (C2'-Me). MS (EI) m/z : 292 [M] $^{+}$ (20), 276 [M-OH] $^{+}$ (77), 233 (100). HRMS (EI): observed, 292.1312. $\text{C}_{16}\text{H}_{20}\text{O}_5$ $^{+}$ requires 292.1311.

1,4-bis(3,4,6-Trimethoxy-2-methylphenyl)buta-1,3-diyne (36)

A 1.29 M solution of BuLi in hexanes (0.80 mL, 1.0 mmol) was added dropwise to a stirred solution of **32** (0.21 g, 1.0 mmol) and anhydrous THF (10 mL) under argon at -78°C . The solution was allowed to warm to room temperature over 1 h, then cooled to -78°C . A solution of 2,3-butanedione (0.05 mL, 0.6 mmol) in dry THF (1 mL) was added dropwise and the reaction mixture was allowed to warm to room temperature. After 24 h the solution was diluted with saturated NH $_4$ Cl (20 mL) and H $_2$ O (50 mL), then extracted with EtOAc (3 \times 15 mL). The extract was washed with brine (30 mL), dried and evaporated to give a brown solid, which was subjected to flash chromatography. Elution with 40% EtOAc/hexanes yielded **36** as a white solid (36 mg, 17%), m.p. = 210–214 $^{\circ}\text{C}$. R $_f$ (40% EtOAc/hexanes): 0.25; IR (KBr) ν_{max} cm $^{-1}$: 2342 & 2140 (C \equiv C). ^1H NMR (600 MHz): δ 6.32 (s, 2H, H5'), 3.89 (s, 6H, OMe), 3.88 (s, 6H, OMe), 3.72 (s, 6H, OMe), 2.40 (s, 6H, C2-Me). ^{13}C NMR (100 MHz) δ 159.0 (C4' or C6'), 154.1 (C4' or C6'), 141.0 (C3'), 136.6 (C2'), 104.0 (C1'), 94.1 (C5'), 80.7 (C1 or C2); 77.6 (C1 or C2), 60.4 (OMe), 56.2 (OMe), 55.8 (OMe), 14.2 (C2-Me). MS (EI) m/z : 410 [M] $^{+}$ (100), 395 [M-Me] $^{+}$ (22). HRMS (EI): observed, 410.1730. $\text{C}_{24}\text{H}_{26}\text{O}_6$ $^{+}$ requires 410.1729.

4,5-Dimethyl-4,5-bis(phenylethynyl)-1,3-dioxolan-2-one (cis/meso **40** and \pm)-trans **41**)

A solution of triphosgene (0.18 g, 0.62 mmol) in DCM (1 mL) was added dropwise to a stirred solution of **38/39** (~1:1 mixture

of diastereomers) 44 (0.20 g, 0.70 mmol) and pyridine (0.17 mL, 2.1 mmol) in DCM (2 mL) under argon at 0°C . The reaction allowed to warm to room temperature slowly. After 2 h the solution was diluted with H $_2$ O (20 mL) and extracted with DCM (3 \times 20 mL). The extract was washed with brine (20 mL), dried and evaporated to give a yellow oil, which was subjected to flash chromatography. Elution with 20% EtOAc/hexanes yielded **41** (42 mg, 19%) as a pale yellow solid, m.p. = 122–127 $^{\circ}\text{C}$. R $_f$ (20% EtOAc/hexanes): 0.35; IR (thin film) ν_{max} cm $^{-1}$: 2252 (C \equiv C), 1803 (C=O). ^1H NMR (400 MHz): δ 7.48 (m, 4H, ArH), 7.38–7.26 (m, 6H, ArH), 2.02 (s, 6H, Me). ^{13}C NMR (100 MHz) δ 152.2 (C2), 131.9 (CH), 129.6 (CH), 128.5 (CH), 120.8 (ArC), 90.4 (ArC \equiv = C2'), 83.0 (C1' or C4/5), 82.6 (C1' or C4/5), 24.9 (Me). MS (EI) m/z : 272 [M-CO $_2$] $^{+}$ (1), 256 [M-CO $_3$] $^{+}$ (18), 128.0 (100). HRMS (ES): observed, 358.1440. $[\text{C}_{21}\text{H}_{17}\text{O}_3 + \text{MeCN}]^{+}$ requires 358.1438.

Further elution gave **40** (62 mg, 28%) as a pale yellow oil. R $_f$ (20% EtOAc/hexanes): 0.25; IR (thin film) ν_{max} cm $^{-1}$: 2230 (C \equiv C), 1815 (C=O). ^1H NMR (400 MHz): δ 7.30 (4H, m, ArH), 7.32–7.38 (2H, m, ArH), 7.29–7.31 (4H, m, ArH), 1.84 (6H, s, Me). ^{13}C NMR (100 MHz) δ 152.3 (C2), 132.0 (PhC), 129.4 (PhC), 128.4 (PhC), 121.9 (PhC), 89.6 (C4 & C5), 84.4 (C1' or C2'), 81.8 (C1' or C2'), 22.5 (Me). MS (EI) m/z : 272 [M-CO $_2$] $^{+}$ (8), 256 [M-CO $_3$] $^{+}$ (28), 128 (100). HRMS (ES): observed, 358.1441. $[\text{C}_{21}\text{H}_{17}\text{O}_3 + \text{MeCN}]^{+}$ requires 358.1438.

3-(3,4,6-Trimethoxy-2-methylphenyl)prop-2-yn-2-ol (43)

A stirred mixture of iodo-1,4,5-trimethoxy-3-methylbenzene (**5**) (4.07 g, 13.2 mmol), CuI (92 mg, 4.0 mol%), Pd(PPh $_3$) $_2$ Cl $_2$ (0.12 g, 1.5 mol%) and DMSO (24 mL) in a Young's flask at 50°C was evacuated and backfilled with argon (x 3). Et $_2$ NH (5 mL, 50 mmol) and prop-2-yn-1-ol (**42**) (2.4 mL, 42 mmol) were added and the vessel sealed. After stirring for 3 d at 50°C fresh additions of CuI (89 mg, 3.9 mol%), Pd(PPh $_3$) $_2$ Cl $_2$ (99 mg, 1.3 mol%) and **42** (1.2 mL, 21 mmol) were made. After a further 24 h at 50°C , the reaction was cooled to room temperature diluted with H $_2$ O (100 mL) and 1M HCl (30 mL). The aqueous solution was extracted with EtOAc (4 \times 30 mL), washed with brine (30 mL), dried and the solvent evaporated to give a brown oil, which was subjected to flash chromatography. Elution with 40% EtOAc/hexanes yielded **43** (2.08 g, 67%) as a white solid, m.p. = 108–114 $^{\circ}\text{C}$. R $_f$ (40% EtOAc/hexanes): 0.17; IR (thin film) ν_{max} cm $^{-1}$: 3000–3600 (OH), 2218 (C \equiv C). ^1H NMR (400 MHz): δ 6.33 (s, 1H, H5'), 4.56 (d, J = 5.6 Hz, 2H, H1), 3.88 (s, OMe3H), 3.86 (s, 3H, OMe), 3.72 (s, 3H, OMe), 2.35 (s, 3H, C2'-Me), 1.79 (t, J = 5.6 Hz, 1H, OH). ^{13}C NMR (100 MHz) δ 157.5 (C4' or C6'), 153.7 (C4' or C6'), 141.1 (C3'), 135.7 (C2'), 104.0 (C1'), 94.2 (C5'), 94.0 (C3); 80.6 (C2), 60.4 (OMe), 56.2 (OMe), 55.8 (OMe), 51.9 (C1), 14.1 (C2'-Me). MS (EI) m/z : 236.0 [M] $^{+}$ (100), 221.0 [M-Me] $^{+}$ (84), 205 [M-MeOH] $^{+}$ (38). HRMS (EI): observed, 236.1044. $\text{C}_{13}\text{H}_{16}\text{O}_4$ $^{+}$ requires 236.1049.

3-(3,4,6-Trimethoxy-2-methylphenyl)propionaldehyde (44)

Method 1: A suspension of activated MnO $_2$ (1.63 g, 18.7 mmol) in a solution of **43** (0.24 g, 1.1 mmol) in DCM (5 mL) was stirred for 16 h. The reaction mixture was vacuum filtered through Celite and washed through with DCM (4 \times 5 mL). The filtrate was

evaporated to yield **44** as a pale yellow solid (0.16 g, 67%) identical with the material described below.

Method 2: Ac₂O (10 mL, 0.11 mol) was added to a stirred solution of **43** (1.24 g, 4.95 mmol) in dry DMSO (40 mL). After 24 h the solution was diluted with H₂O (60 mL) and NEt₃ (10 mL), then extracted with EtOAc (4 × 20 mL). The extract was washed with brine (20 mL), dried and evaporated to yield **44** as a pale yellow solid (1.21 g, quant.), m.p. = 126–129 °C. R_f (40% EtOAc/hexanes): 0.3; IR (thin film) ν_{max} cm⁻¹: 2166 (C≡C), 1645 (C=O). ¹H NMR (600 MHz): δ 9.46 (s, 1H, CHO), 6.33 (s, 1H, H5'), 3.92 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.73 (s, 3H, OMe), 2.39 (s, 3H, C2'-Me). ¹³C NMR (150 MHz) δ 176.8 (C=O), 160.4 (C6' or C4'), 156.9 (C6' or C4'), 141.4 (C3'), 137.9 (C2'), 101.0 (C1'), 96.9 (C2 or C3), 93.9 (C5'), 92.9 (C2 or C3), 60.7 (OMe); 56.3 (OMe), 56.0 (OMe), 14.3 (C2'-Me). MS (EI) *m/z*: 234 [M]⁺ (100), 219.0 [M-Me]⁺ (42). HRMS (EI): observed, 234.0898. C₁₃H₁₄O₄⁺ requires 234.0892.

1,6-bis(3,4,6-Trimethoxy-2-methylphenyl)hexa-1,5-diyne-3,4-diol (45) (~13:20 mixture of diastereomers based on 46)

Freshly prepared Cu/Zn couple⁴⁵ (3.1 g, 1:1 - CuSO₄·5H₂O:Zn w/w) and AcOH (1 mL, 20 mmol) were added to a stirred solution of **44** (1.01 g, 4.28 mmol) in THF (30 mL). After 3 d the reaction mixture was diluted with H₂O (100 mL) and saturated NaHCO₃ (10 mL) then vacuum filtered through Celite and washed through with DCM (3 × 10 mL). The filtrate was concentrated under reduced pressure to ~100 mL and the resulting precipitate was collected by vacuum filtration and air-dried, giving **45** as a pale yellow solid (0.82 g, 82%), m.p. = 192–195 °C. R_f (70% EtOAc/hexanes): 0.25; IR (thin film) ν_{max} cm⁻¹: 3000–3500 (OH), 2222 (C≡C). ¹H NMR (400 MHz): δ 6.31 (s, 2H, H5'), 4.81 (d, *J* = 6.4 Hz, 2H, H3/H4), 3.87 (s, 6H, OMe), 3.82 (s, 6H, OMe), 3.69 (s, 6H, OMe), 3.01 (d, *J* = 6.4 Hz, 2H, OH), 2.31 (s, 6H, C2'-Me). ¹³C NMR (100 MHz) δ 158.1 (C4' or C6'), 154.2 (C4' or C6'), 141.3 (C3'), 135.9 (C2'), 104.0 (C1'), 94.4 (C5'), 93.3 (C1 or C2); 82.5 (C1 or C2), 68.2 (C3 & C4), 60.7 (OMe), 56.5 (OMe), 56.1 (OMe), 14.4 (C2'-Me). MS (EI) *m/z*: 455 [M-Me]⁺ (25), 453 [M-OH]⁺ (71). HRMS (EI): observed, 470.1937. C₂₆H₃₀O₈⁺ requires 470.1941.

4,5-bis((3,4,6-Trimethoxy-2-methylphenyl)ethynyl)-1,3-dioxolan-2-one (46) (~13:20 mixture of diastereomers)

A solution of triphosgene (0.19 g, 0.67 mmol) in DCM (2 mL) was added dropwise to a stirred solution of **45** (mixture of diastereomers) (32 mg, 68 μmol) and pyridine (0.10 mL, 1.3 mmol) in DCM (6 mL) under argon at 0 °C. The reaction mixture was allowed to warm to room temperature slowly. After 45 min the solution was diluted with H₂O (20 mL) and extracted with DCM (3 × 10 mL). The extract was washed with brine (20 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 60% EtOAc/hexanes gave **46** as a pale yellow solid (15 mg, 45%), m.p. = 165–170 °C. IR (thin film) ν_{max} cm⁻¹: 2226 (C≡C), 1810 (C=O). MS (ES) *m/z*: 515 [M+H+H₂O]⁺ (50), 497 [M+H]⁺ (100), 471 [M+H-CO]⁺ (18), 453 [M+H-CO₂]⁺ (89). HRMS (ES): observed 497.1821. C₂₇H₂₉O₉⁺ requires 497.1806.

Isomer 1 (major): R_f (60% EtOAc/hexanes): 0.35. ¹H NMR

CDCl₃ (400 MHz) δ 6.33 (s, 2H, H5'), 5.59 (2H, s, H4/5), 3.89 (s, 6H, OMe), 3.86 (s, 6H, OMe), 3.72 (s, 6H, C3'-OMe), 2.34 (s, 6H, C2'-Me). ¹³C NMR CDCl₃ (150 MHz) δ 158.2 (C4' or C6'), 155.0 (C4' or C6'), 153.3 (C2), 141.2 (C3'), 136.5 (C2'), 102.2 (C1'), 94.21 (C5'), 87.7 (C1'' or C2''), 86.32 (C1'' or C2''), 73.4 (C4/5), 60.6 (C3'-OMe), 56.3 (OMe), 56.0 (OMe), 14.2 (C2'-Me).

Isomer 2 (minor): R_f (60% EtOAc/hexanes): 0.3; ¹H NMR CDCl₃ (400 MHz) δ 6.28 (s, 2H, H5'), 5.77 (s, 2H, H4/5), 3.87 (s, 6H, OMe), 3.73 (s, 6H, OMe), 3.66 (s, 6H, C3'-OMe), 2.24 (s, 6H, C2'-Me). ¹³C NMR CDCl₃ (100 MHz) δ 158.2 (C4' or C6'), 154.4 (C4' or C6'), 153.1 (C2), 140.8 (C3'), 136.2 (C2'), 102.4 (C1'), 93.7 (C5'), 87.5 (C1'' or C2''), 85.9 (C1'' or C2''), 71.3 (C4/5), 60.2 (C3'-OMe), 55.8 (OMe), 55.6 (OMe), 13.8 (C2'-Me).

1,6-bis(3,4,6-Trimethoxy-2-methylphenyl)hexa-1,5-diyne-3,4-dione (47)

Ac₂O (5 mL, 0.05 mol) was added to a stirred solution of **45** (0.69 g, 1.5 mmol) and DMSO (20 mL). After 5 h the reaction mixture was cooled to 0 °C and diluted with H₂O (50 mL). The resulting precipitate was vacuum filtered to give an orange solid. The filtrate was diluted with 28% aqueous NH₃ (8 mL), then extracted with EtOAc (3 × 20 mL). The extract was washed with brine (20 mL), dried and evaporated and combined with the precipitate to give **47** as an orange solid (0.66 g, 90%), m.p. = 231–235 °C. R_f (60% EtOAc/hexanes): 0.35; IR (thin film) ν_{max} cm⁻¹: 2177 (C≡C), 1662 (C=O). ¹H NMR (600 MHz): δ 6.31 (s, 2H, H5'), 3.93 (s, 6H, OMe), 3.88 (s, 6H, OMe), 3.73 (s, 6H, OMe), 2.47 (s, 6H, C2'-Me). ¹³C NMR (150 MHz) δ 173.3 (C=O), 161.1 (C4' or C6'), 157.3 (C4' or C6'), 141.3 (C3'), 138.6 (C2'), 100.8 (C1'), 98.0 (C1/6), 94.8 (C2/5); 93.7 (C5'), 60.5 (OMe), 56.3 (OMe), 55.9 (OMe), 14.2 (C2'-Me). HRMS (ES): observed, 467.1702. C₂₆H₂₇O₈⁺ requires 467.1706.

Attempted pinacol coupling of antrocamphin B (3) with Zn/Cu couple

Freshly prepared Cu/Zn couple⁴⁵ (3.01 g, 1:1 - CuSO₄·5H₂O:Zn w/w) and AcOH (1.5 mL, 26 mmol) were added to a stirred solution of antrocamphin B (**3**) (1.21 g, 4.88 mmol) in THF (40 mL). After 30 h the reaction suspension was vacuum filtered through Celite and washed through with DCM (3 × 15 mL). The filtrate was evaporated residue was subjected to flash chromatography. Elution with 40% EtOAc/hexanes gave **meso-4,5-bis(3,4,6-trimethoxy-2-methylphenyl)octane-2,7-dione (50)** as a pale yellow solid (92 mg, 8%), m.p. = 203–206 °C. R_f (60% EtOAc/hexanes): 0.35; IR (thin film) ν_{max} cm⁻¹: 1710 (C=O). ¹H NMR (400 MHz): δ 6.35 (s, 2H, H5'), 4.20–4.22 (m, 2H, H3/6 or H4/5), 3.88 (s, 6H, OMe), 3.84 (s, 6H, OMe), 3.71 (s, 6H, OMe), 2.97–3.03 (m, 2H, C3/6 or C4/5), 2.43 (s, 6H, C2'-Me), 2.22–2.27 (m, 2H, H3/6 or H4/5). ¹³C NMR (100 MHz) δ 209.2 (C2/7'), 154.8 (C4' or C6'), 151.6 (C4' or C6'), 141.1 (C3'), 133.1 (C2'), 122.3 (C1'), 95.3 (C5'), 60.6 (OMe), 55.8 (OMe), 55.7 (OMe), 45.9 (C3/6 or C4/5), 37.3 (C3/6 or C4/5), 30.5 (C1/8), 14.2 (C2'-Me). MS (ES) *m/z*: 541 [M+K]⁺, 525 [M+Na]⁺. HRMS (ES): observed 503.2626. C₂₈H₃₉O₈⁺ requires 503.2639.

Further elution with 60% EtOAc/hexanes gave **3,4-dimethyl-1,6-bis(3,4,6-trimethoxy-2-methylphenyl)hexa-1,5-diene-3,4-diol**

(**49**) as a white solid (0.28 g, 22%), m.p. = 138–141 °C. R_f (60% EtOAc/hexanes): 0.30; IR (thin film) ν_{\max} cm^{-1} : 3200–3600 (OH). ^1H NMR (600 MHz): δ 6.58 (d, J = 16.2 Hz, 2H, H6), 6.36 (s, 2H, H5'), 6.26 (d, J = 16.2 Hz, 2H, H5), 3.86 (s, 6H, OMe), 3.71 (s, 12H, 2 \times OMe), 2.48 (s, 6H, OH), 2.25 (s, 6H, C2'-Me), 1.45 (s, 6H, H3/4-Me). ^{13}C NMR (100 MHz) δ 154.0 (C4' or C6'), 151.9 (C4' or C6'), 141.5 (C3'), 137.9 (C6), 131.3 (C1' or C2'), 122.9 (C5), 118.7 (C1' or C2'), 95.1 (C5'), 78.2 (C4), 60.6 (OMe), 56.0 (OMe), 22.4 (C4-Me), 13.4 (C2'-Me). MS (ES) m/z : 541 [M+K]⁺ (100), 539 [M+K-H₂]⁺ (20), 525 [M+Na]⁺ (50), 523 [M+Na-H₂]⁺ (35), 507 [M+Na-H₂O] (10). HRMS (ES): observed 525.2468. C₂₈H₃₈O₈Na⁺ requires 525.2459.

3,4-Dimethyl-1,6-bis(3,4,6-trimethoxy-2-methylphenyl)hexa-1,5-diyne-3,4-diol (**34**)

A flame dried Schlenk flask under argon was charged with samarium metal (75 mg, 0.49 mmol), 1,2-diiodoethane (65 mg, 0.23 mmol) and dry THF (4 mL), purging and back-filling with argon after each addition. After 30 min of stirring the reaction mixture containing SmI₂ was treated with a solution of antrocamphin B (**3**) (63 mg, 0.25 mmol) in dry THF (1 mL). After 2.5 h the reaction was quenched with H₂O (20 mL) and the aqueous solution was extracted with EtOAc (3 \times 10 mL). The extract was washed with brine (10 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 60% EtOAc/hexanes gave **34** (10:1 mixture of *rac:meso* isomers) (35 mg, 55%) as a pale yellow solid. IR (thin film) ν_{\max} cm^{-1} : 3100–3700 (OH), 2222 (C \equiv C).

The isomers were separated by semi-preparative HPLC using a Hewlett Packard 1050 system equipped with a multiple wavelength detector (MWD). Separation was achieved using a 250 \times 10 mm i.d., 5 μm , Apollo C18 reversed phase column (Grace-Davison) with a 33 mm \times 7 mm guard column of the same material. The column was eluted at 4 mL.min⁻¹ with 30% (v/v) acetonitrile–water and 1 mL was injected. The chiral isomers eluted first giving **34b** as a white solid: m.p. = 126–130 °C. R_f (60% EtOAc/hexanes): 0.2. ^1H NMR CDCl₃ (600 MHz) δ 6.30 (s, 2H, H5'), 3.86 (s, 6H, OMe), 3.81 (s, 6H, OMe), 3.69 (s, 6H, OMe), 3.37 (s, 2H, OH), 2.33 (s, 6H, C2'-Me), 1.72 (6H, s, C3/4-Me). ^{13}C NMR CDCl₃ (150 MHz) δ 157.9 (C4' or C6'), 153.8 (C4' or C6'), 141.2 (C3'), 135.2 (C2'), 104.3 (C1'), 97.3 (C1/6 or C2/5), 94.5 (C5'), 80.6 (C1/6 or C2/5), 75.3 (C3/4), 60.6 (OMe), 56.4 (OMe), 56.0 (OMe), 23.9 (C3/4-Me), 14.3 (C2'-Me).

Further elution gave the *meso* isomer **34a** as a white solid: m.p. = 138–142 °C. R_f (60% EtOAc/hexanes): 0.2. ^1H NMR CDCl₃ (600 MHz) δ 6.29 (s, 2H, H5'), 3.86 (s, 6H, OMe), 3.79 (s, 6H, OMe), 3.69 (s, 6H, OMe), 3.23 (s, 2H, OH), 2.29 (s, 6H, C2'-Me), 1.72 (s, 6H, C3/4-Me). ^{13}C NMR CDCl₃ (150 MHz) δ 157.9 (C4' or C6'), 153.7 (C4' or C6'), 141.2 (C3'), 135.4 (C2'), 104.4 (C1'), 97.9 (C1/6 or C2/5), 94.5 (C5'), 80.4 (C1/6 or C2/5), 74.9 (C3/4), 60.5 (OMe), 56.4 (OMe), 55.9 (OMe), 22.9 (C3/4-Me), 14.2 (C2'-Me). MS (ES) m/z : 537 [M+K]⁺ (18), 521 [M+Na]⁺ (80), 481 [M-OH]⁺ (40). HRMS (ES): observed 499.2338. C₂₈H₃₅O₈⁺ requires 499.2326.

(\pm)-*trans*-4,5-Dimethyl-4,5-bis((3,4,6-trimethoxy-2-methylphenyl)ethynyl)-1,3-dioxolan-2-one (**51**)

Method 1: A solution of triphosgene (0.13 g, 0.42 mmol) in DCM (2 mL) was added dropwise to a stirred solution of **34** (10:1 *rac:meso* mixture) (0.18 g, 0.36 mmol) and pyridine (0.10 mL, 1.3 mmol) in DCM (8 mL) under argon at 0 °C, then the reaction was allowed to warm to room temperature slowly. After 4 h the solution was diluted with H₂O (30 mL) and extracted with DCM (3 \times 20 mL). The extract was washed with brine (20 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 60% EtOAc/hexanes gave **51** (30 mg, 16%) as a pale yellow/orange solid, m.p. = 85–90 °C. R_f (40% EtOAc/hexanes): 0.35; IR (thin film) ν_{\max} 2207 (C \equiv C), 1787 (C=O). ^1H NMR CDCl₃ (600 MHz) δ 6.33 (s, 2H, H5'), 3.87 (s, 6H, OMe), 3.85 (s, 6H, OMe), 3.72 (s, 6H, OMe), 2.36 (s, 6H, C2'-Me), 1.70 (s, 6H, C4/5-Me). ^{13}C NMR CDCl₃ (150 MHz) δ 158.5 (C4' or C6'), 154.7 (C4' or C6'), 153.0 (C=O), 141.2 (C3'), 135.8 (C2'), 102.9 (C1'), 94.4 (C5'), 90.1 (C4/5 or C1"), 86.0 (C2"), 83.5 (C4/5 or C1"), 60.6 (OMe), 56.4 (OMe), 56.0 (OMe), 25.5 (C4/5-Me), 14.27 (C2'-Me). HRMS (ES): observed 525.2132. C₂₉H₃₃O₉⁺ requires 525.2125.

Method 2: A flame dried Schlenk flask under argon was charged sequentially with samarium metal (76 mg, 0.51 mmol), 1,2-diiodoethane (0.13 g, 0.47 mmol) and dry THF (4 mL), purging and back-filling with argon after each addition. After 30 min of stirring a solution of antrocamphin B (**3**) (69 mg, 0.28 mmol) in dry THF (1 mL) was added via cannula to the preformed solution of SmI₂. After 1.5 h the reaction mixture was quenched with methyl chloroformate (30 μL , 0.39 mmol) and the reaction mixture was stirred for a further 30 min before being diluted with H₂O (20 mL) and extracted with EtOAc (3 \times 10 mL). The extract was washed with brine (10 mL), dried and evaporated, and the residue was subjected to flash chromatography. Elution with 40% EtOAc/hexanes gave **51** as a pale yellow solid (23 mg, 32%) identical with the material described above.

TNF expression inhibition assays

These were conducted as described previously.³²

Acknowledgements

We acknowledge the facilities and scientific and technical assistance of the Australian Microscopy and Microanalysis Research Facility at the Centre for Microscopy, Characterisation, and Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments. MRB is, and KAP was, the recipient of an Australian Postgraduate Award. We thank Professors Mark Ogden and George Koutsantonis for valuable discussions regarding alkyne-Pd complexation.

Notes and references

¹¹⁰ ^a School of Chemistry and Biochemistry, The University of Western Australia, Perth, WA, Australia. Fax: +618 6488 1005; Tel: +61 8 6488 3170; E-mail: matthew.piggott@uwa.edu.au

^b Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Perth, WA, Australia.

- † Electronic Supplementary Information (ESI) available: ^1H and ^{13}C NMR spectra of all new compounds and crystallographic data. Full CIF depositions (excluding structure factor amplitudes) have been deposited with the Cambridge Crystallographic Data Centre (CCDC no's 970520 (50) and 970521 (51)). These data can be obtained free of charge through www.ccdc.cam.ac.uk. See DOI: 10.1039/b000000x/
- J.-J. Chen, W.-J. Lin, C.-H. Liao and P.-C. Shieh, *J. Nat. Prod.*, 2007, **70**, 989-992.
 - K. A. Punch, E. L. Ghisalberti and M. J. Piggott, *J. Nat. Prod.*, 2011, **74**, 1348-1350.
 - Y.-H. Hsieh, F.-H. Chu, Y.-S. Wang, S.-C. Chien, S.-T. Chang, J.-F. Shaw, C.-Y. Chen, W.-W. Hsiao, Y.-H. Kuo and S.-Y. Wang, *J. Agric. Food Chem.*, 2010, **58**, 3153-3158.
 - L.-S. Shi, C.-H. Chao, D.-Y. Shen, H.-H. Chan, C.-H. Chen, Y.-R. Liao, S.-J. Wu, Y.-L. Leu, Y.-C. Shen, Y.-H. Kuo, E. J. Lee, K. Qian, T.-S. Wu and K.-H. Lee, *Bioorg. Med. Chem.*, 2011, **19**, 677-683.
 - C.-L. Lee, C.-H. Huang, H.-C. Wang, D.-W. Chuang, M.-J. Wu, S.-Y. Wang, T.-L. Hwang, C.-C. Wu, Y.-L. Chen, F.-R. Chang and Y.-C. Wu, *Org. Biomol. Chem.*, 2011, **9**, 70-73.
 - M. V. Sargent and P. O. Stransky, *J. of the Chem. Soc., Perkin Trans. 1*, 1982, 2373-2377.
 - M. Matsumoto, K. Kobayashi and Y. Hotta, *J. Org. Chem.*, 1984, **49**, 4740-4741.
 - A.-S. Castanet, F. Colobert and P.-E. Broutin, *Tetrahedron Lett.*, 2002, **43**, 5047-5048.
 - H. Firouzabadi, N. Iranpoor and S. Kazemi, *Can J. Chem.*, 2009, **87**, 1675.
 - R. D. Stephens and C. E. Castro, *J. Org. Chem.*, 1963, **28**, 3313-3315.
 - F. G. Schreiber and R. Stephenson, *J. Chem. Soc., Perkin Trans. 1: Org. and Bio-Org. Chem. (1972-1999)*, 1977, 90-92.
 - Y.-R. Liao, P.-C. Kuo, J.-W. Liang, Y.-C. Shen and T.-S. Wu, *Int. J. Mol. Sci.*, 2012, **13**, 10432-10440.
 - A. Padwa and G. S. K. Wong, *J. Org. Chem.*, 1986, **51**, 3125.
 - E. D. Goddard-Borger, E. L. Ghisalberti and R. V. Stick, *Euro. J. Org. Chem.*, 2007, 3925-3934.
 - S. Kobayashi and T. Kobayashi, *Molecules*, 2000, **5**, 1062.
 - I. V. Alabugin, V. I. Timokhin, J. N. Abrams, M. Manoharan, R. Abrams and I. Ghiviriga, *J. Am. Chem. Soc.*, 2008, **130**, 10984.
 - Y. Yamamoto, A. Nagata, Y. Arikawa, K. Tatsumi and K. Itoh, *Organometallics*, 2000, **19**, 2403-2405.
 - H. S. Blanchette, S. C. Brand, H. Naruse, T. J. R. Weakley and M. M. Haley, *Tetrahedron*, 2000, **56**, 9581-9588.
 - Y.-T. He, S. Xue, T.-S. Hu and Z.-J. Yao, *Tetrahedron Lett.*, 2005, **46**, 5393-5397.
 - J. G. Rodriguez and T. Laparra, *Tetrahedron*, 2009, **65**, 2551.
 - R. B. Davis and P. Hurd, *J. Am. Chem. Soc.*, 1954, **77**, 3284.
 - F. J. Wilson and W. M. Hyslop, *J. Chem. Soc.*, 1923, **123**, 2612.
 - A. Boussonniere, R. Beneteau, N. Zimmerman, J. Lebreton and F. Denes, *Chem. Euro. J.*, 2011, **17**, 5613.
 - T. Ishikawa, T. Mizuta, K. Hagiwara, T. Aikawa, T. Kudo and S. Saito, *J. Org. Chem.*, 2002, **68**, 3702.
 - D. Crich, A. B. Pavlovic and D. J. Wink, *Synth. Commun.*, 1999, **29**, 359.
 - R. Brettle and S. M. Shibib, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2912.
 - C. Clausen, R. Wartchow and H. Butenschon, *Euro. J. Org. Chem.*, 2001, 93.
 - B. Guerin and L. J. Johnston, *J. Org. Chem.*, 1989, **54**, 3176-3181.
 - H. Zhao, D. J. Li, L. Liu and Q. X. Guo, *Chem. Commun.*, 2003, 506.
 - T. Tsuritani, S. Ito, H. Shinokubo and K. Oshima, *J. Org. Chem.*, 2000, **65**, 5066.
 - L. Lu, J. M. Fang, G. H. Lee and W. Yu, *J. Chin. Chem. Soc.*, 1997, **44**, 279.
 - S. G. Stewart, C. J. Braun, S.-L. Ng, M. E. Polomska, M. Karimi and L. J. Abraham, *Bioorg. Med. Chem.*, 2010, **18**, 650-662.
 - S. G. Stewart, D. Spagnolo, M. E. Polomska, M. Sin, M. Karimi and L. J. Abraham, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5819-5824.
 - S. Y. Yeung, S. Kampmann, K. A. Stubbs, B. W. Skelton, B. J. Kaskow, L. J. Abraham and S. G. Stewart, *MedChemComm*, 2011, **2**, 1073-1078.
 - M. Karimi, L. C. Goldie, M. N. Cruickshank, E. K. Moses and L. J. Abraham, *Euro. J. Hum. Genet.*, 2009, **17**, 1454-1462.
 - M. Karimi, L. C. Goldie, D. Ulgiati and L. J. Abraham, *BioTechniques*, 2007, **42**, 217-224.
 - M. Buccini, S. Y. Jeow, L. Byrne, B. W. Skelton, T. M. Nguyen, C. L. L. Chai and M. J. Piggott, *Euro. J. Org. Chem.*, 2013, **2013**, 3232-3240.
 - G. M. Sheldrick, *Acta Crystallogr. A*, 2008, **64**, 112-122.
 - P. Wang, Z. Zhang and B. Yu, *J. Org. Chem.*, 2005, **70**, 8884-8889.
 - I. M. Godfrey, M. V. Sargent and J. A. Elix, *J. Chem. Soc., Perkin Trans. 1*, 1974, **11**, 1353-1354.
 - J. A. Elix and V. J. Portelli, *Aust. J. Chem.*, 1990, **43**, 1773.
 - F. G. Schreiber and R. Stephenson, *J. Chem. Soc., Perkin Trans. 1*, 1977, 90-92.
 - I. V. Alabugin, V. I. Timokhin, J. N. Abrams, M. Manoharan, R. Abrams and I. Ghiviriga, *J. Am. Chem. Soc.*, 2008, **130**, 10984-10995.
 - F. J. Wilson and W. M. Hyslop, *J. Chem. Soc.*, 1923, **123**, 2612-2618.
 - R. S. Shank and H. Shechter, *J. Org. Chem.*, 1959, **24**, 1825-1826.