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Ethynylbenzenoid metabolites of *Antrodia camphorata*: synthesis and inhibition of TNF expression†

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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-mediated 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] (Fig. 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 $CDCl_3$, 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 (Fig. 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.

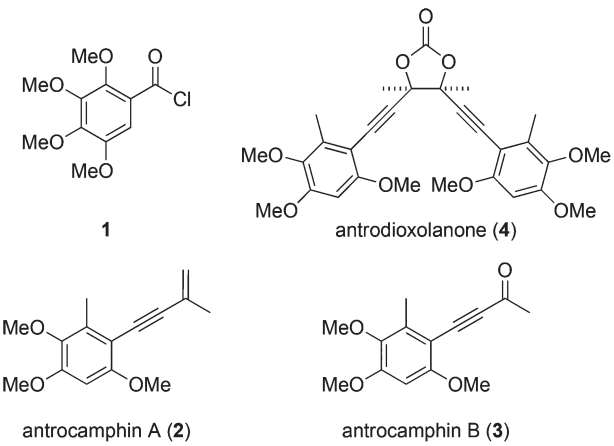

Fig. 1 Benzenoid metabolites reportedly isolated from *Antrodia camphorata*.[1]

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.

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

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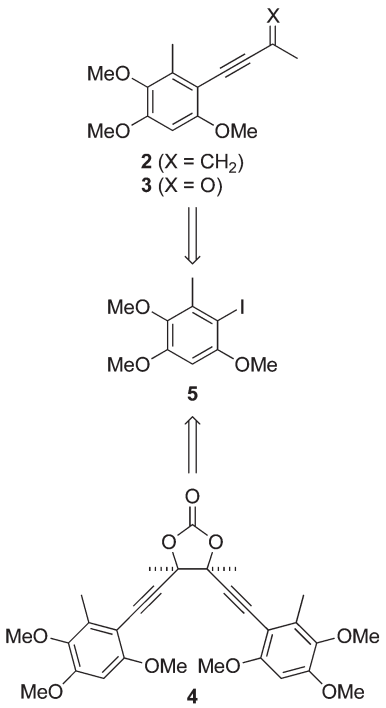
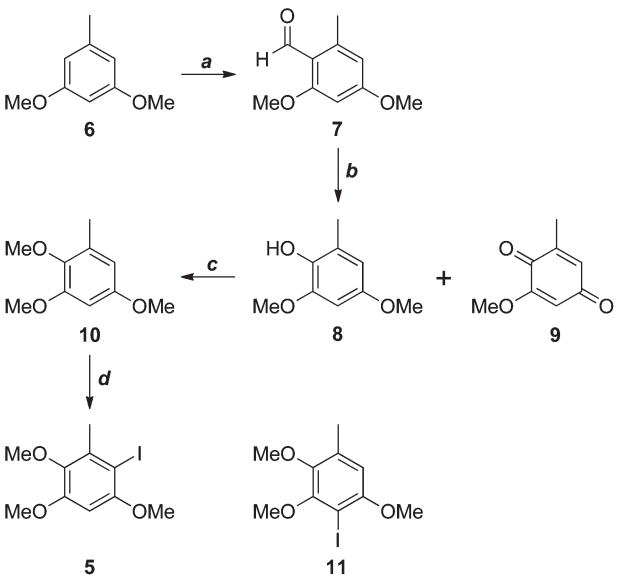
† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR spectra of all new compounds and crystallographic data. CCDC 970520 for **50** and 970521 for **51**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob42333f



Scheme 1 Retrosynthetic analysis.

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 oxidation[7] 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 avoided by keeping reaction times short. Methylation of **8** then provided **10**. Treatment with *N*-iodosuccinimide and catalytic trifluoroacetic acid[8] furnished a quantitative yield of the desired iodide **5**. Alternatively, NaI/Oxone[9] 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**.



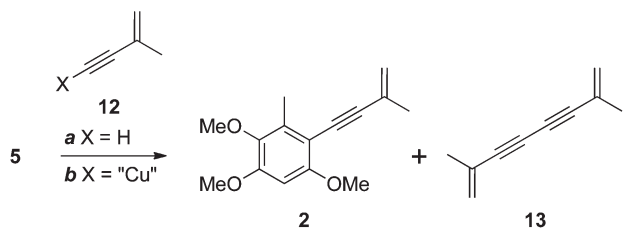
Scheme 2 Reagents, conditions and yields: (a) $POCl_3$, DMF, 97%; (b) H_2O_2, H_2SO_4, MeOH, 97% (optimised for **8**); (c) MeI, K_2CO_3, DMF, 96%; (d) NIS, TFA, MeCN, quant.; or I_2, Oxone, H_2O, 77%.

Synthesis of the antrocamphins

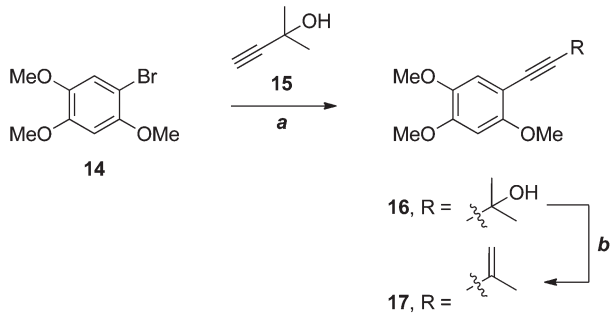
Wu and colleagues completed their total synthesis of antrocamphin A with a low-yielding (10%) Sonogashira coupling of iodide **5** with enyne **12** (X = H, Scheme 3).[5] We also encountered problems with this reaction. Complete conversion to antrocamphin A (**2**) was not achieved despite varying the 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 ^{1}H 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 reaction[10] 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 product[1] and previously synthesised material.[5]

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]

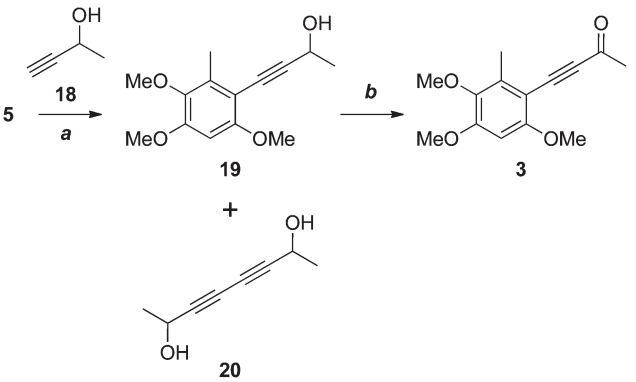
Our attention now turned to antrocamphin B (**3**), which could in principle be derived from the Sonogashira coupling of the iodide **5** with 3-butyne-2-one. However, electron deficient alkynes are poor substrates for the Sonogashira reaction, so we opted for two-step coupling of the propargyl alcohol **18**, followed by oxidation (Scheme 5). After some



Scheme 3 Reagents, conditions and yields: (a) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, MeCN, 80 °C, sealed tube, 44% (as a mixture with **5**); (b) pyridine, reflux, 74% (**2**).



Scheme 4 Wu's synthesis of benzocamphorin F.[12] (a) $Pd(PPh_3)_4$, CuI, DMF, 85%; (b) MsCl, PhMe, microwave, 92%.

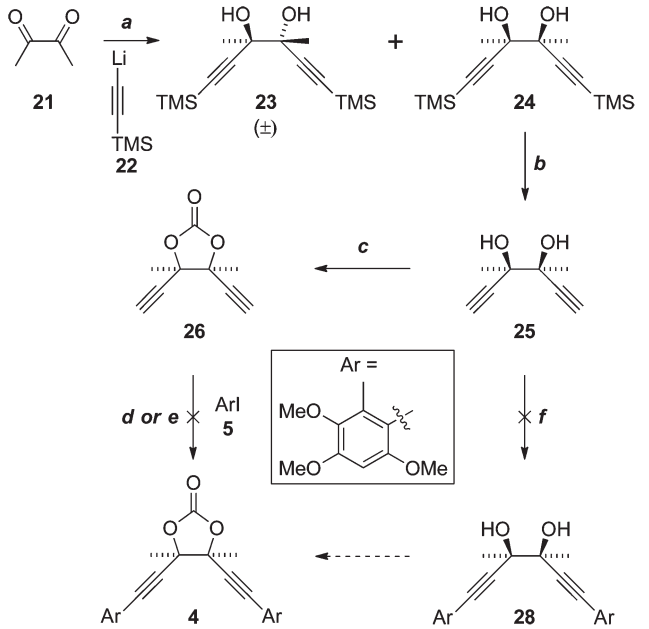


Scheme 5 Reagents, conditions and yields: (a) $Pd(PPh_3)_2Cl_2$, CuI, $HNEt_2$, DMSO, 75 °C, 58%; (b) MnO_2, DCM, 80%; or, DMSO, Ac_2O, quant.

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 surprisingly, the Castro–Stephens reaction of the copper acetylide derived from **18** failed in this case. Oxidation of **19** with MnO_2[13] 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]

Approaches towards antrodioxolanone

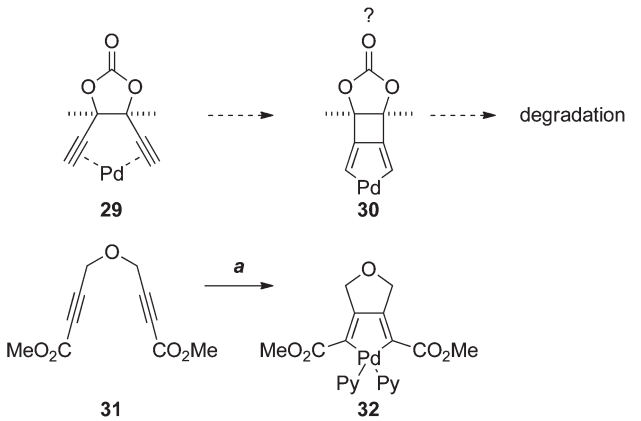
Application of a key double Sonogashira reaction to the synthesis of the more complex antrodioxolanone (**4**) required the *meso*-diol **25**, which was prepared in two steps from diacetyl (**21**) and lithium TMS-acetylide (**22**), as described previously (Scheme 6).[16] Cyclocondensation with triphosgene 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–Stephens 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



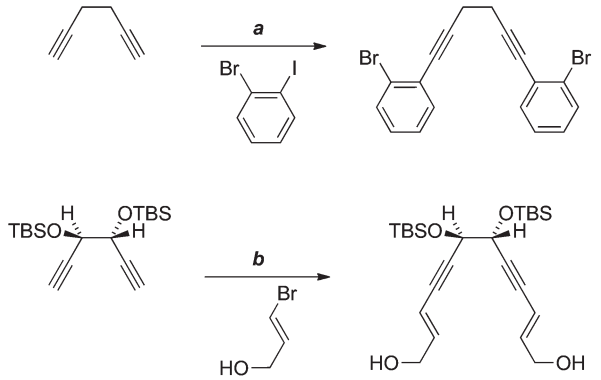
Scheme 6 Reagent, conditions and yields: (a) see ref. 16; (b) K_2CO_3, DCM–MeOH, 96%; (c) $CO(OCCl_3)_2$, pyridine, THF, 83%; (d) $Pd(PPh_3)_2Cl_2$; NEt_3 thin film, or with MeCN, or $HNEt_2$–MeCN, all 0%; (e) 1. $HONH_3Cl$, $CuSO_4{\cdot}5H_2O$, NH_3, H_2O, EtOH (52% based on diacetylide); 2. **5**, pyridine, reflux (0%); (f) $Pd(PPh_3)_2Cl_2$, NEt_3 or $HNEt_2$, MeCN, both 0%.

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–Stephens reactions of **26**, and its instability under the reaction conditions, was puzzling. Such reactions of 1,5-diynes 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).



Scheme 7 Reagents, conditions (a) 1. $Pd_2(dba)_3{\cdot}CHCl_3$, acetone; 2. pyridine (Py), DCM.[17]



Scheme 8 Reagents, conditions and yields: (a) $PdCl_2(PPh_3)_2$, CuI, NEt_3, piperidine, 46%;[18] (b) $PdCl_2(PPh_3)_2$, CuI, $HNEt_2$, 79%.[19]

Although 3-palladabicyclo[3.2.0]hepta-1,4-dienes such as **30** appear to be unprecedented, the [3.3.0]-palladabicycle **32**, synthesised by oxidative addition to the diene **31**, has been isolated and characterised spectroscopically.[17]

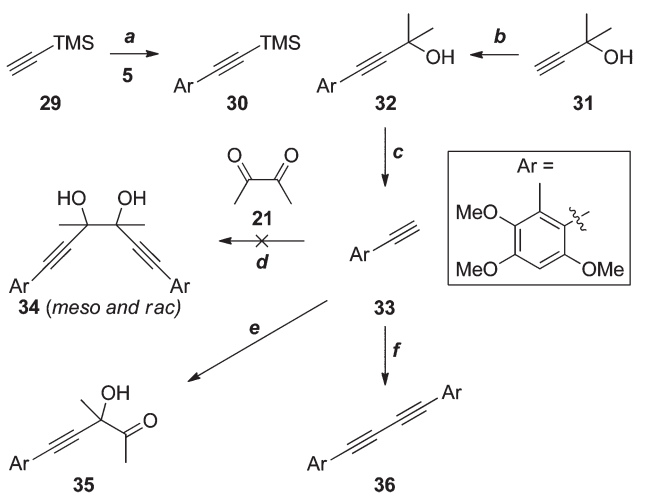
There are just two reported examples of the double Sonogashira reaction of a 1,5-diyne with bridging sp^3-hybridised carbon atoms (Scheme 8).[18,19] These precedents suggested that, for our purposes, it may be possible to effect the sp–sp^2 coupling prior to formation of the dioxolane, that is, with 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.

Given the apparent incompatibility of the diynes **25** and **26** with Sonogashira coupling conditions, out 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 = 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 deprotection[20] of **32** proceeded smoothly to provide the terminal acetylene **33** in excellent yield.

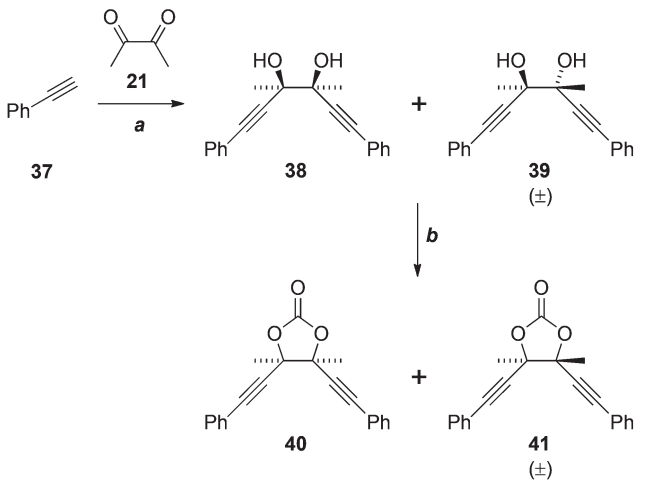
Several attempts at addition of the acetylide, generated *in situ* by deprotonation of **33** with sodium[21] or Grignard reagents,[22] to diacetyl (**21**), resulted in complex mixtures of products, with none of the glycol **34** detected. On one occasion, the mono-addition product **35** was isolated in low yield. When BuLi[23] or tetrapropylammonium hydroxide[24] 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 these reactions. However, an attempted reaction of the less basic cerium acetylide[25] 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** → **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 (Scheme 10), 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.

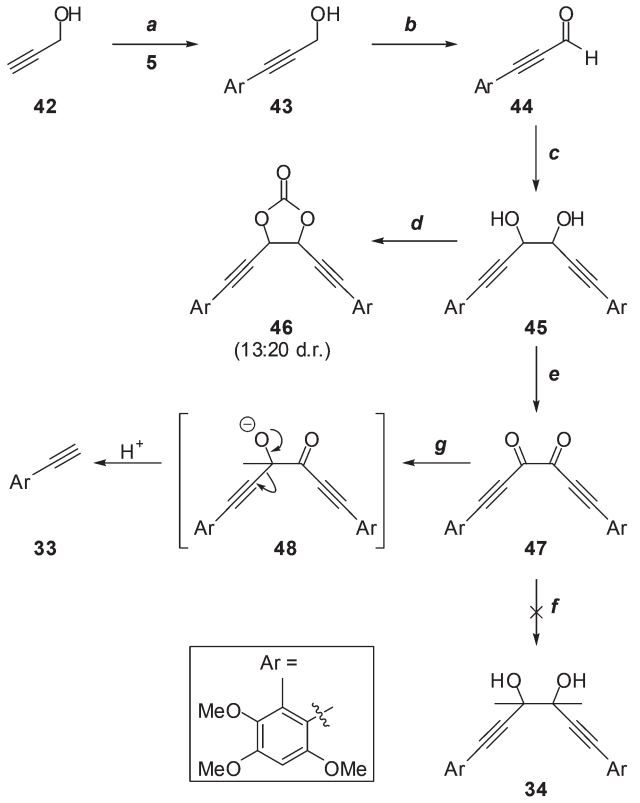
Given the possible complication of deprotonation of diacetyl by acetylide nucleophiles, we investigated the alternative addition of more reactive methylmetallic nucleophiles to dione **47** (Scheme 11), which lacks appreciably acidic protons. Thus, the Sonogashira reaction of propargyl alcohol (**42**) with iodide



Scheme 9 Reagents, conditions and yields: (a) $PdCl_2(PPh_3)_2$, CuI, NEt_3, 21%;(b) $PdCl_2(PPh_3)_2$, 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 = Et or i-Pr. All gave complex mixtures); (e) EtMgBr, Et_2O, then **21**, 11%; (f) BuLi, THF, then **21**, 17%; *or* PrN_4OH, DMSO, **21**, 10%.



Scheme 10 Reagents, conditions and yields: (a) EtMgBr, Et_2O, then **21**, 68% (1 : 1 mixture of **38** and **39**); (f) $CO(OCCl_3)_2$, pyridine, THF, 28% (**40**), 19% (**41**).



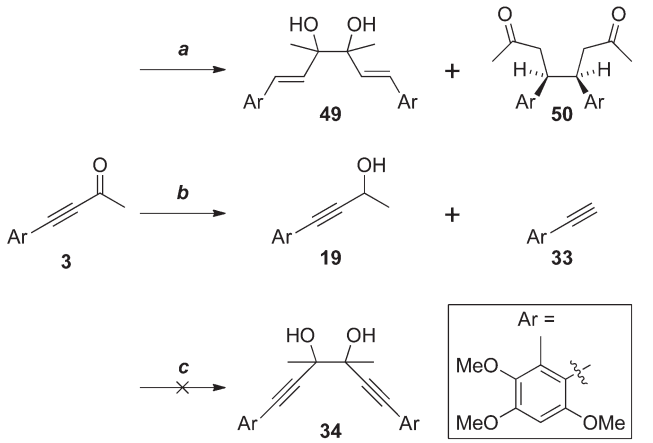
Scheme 11 Reagents, conditions and yields: (a) $PdCl_2(PPh_3)_2$, CuI, $HNEt_2$, DMSO, 67%; (b) MnO_2, DCM, 67% *or* Ac_2O, DMSO, 55%; (c) Cu/Zn, AcOH, THF, 82%; (d) $CO(OCCl_3)_2$, pyridine, DCM, 45%; (e) MnO_2, DCM, 14% (+30% **44**) *or* Ac_2O, DMSO, 99%; (f) MeMgBr, Et_2O and or THF, 0%; MeLi, Et_2O–THF, 50% (**33**).

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 ^{1}H 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 ^{1}H 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 iodide[26] 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.

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 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 coupling[29] 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



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.

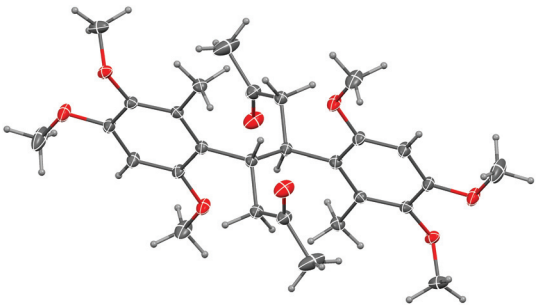


Fig. 2 Representation of the crystal structure of **50**. Ellipsoids are shown at 50% probability amplitudes with hydrogen atoms assigned arbitrary radii.

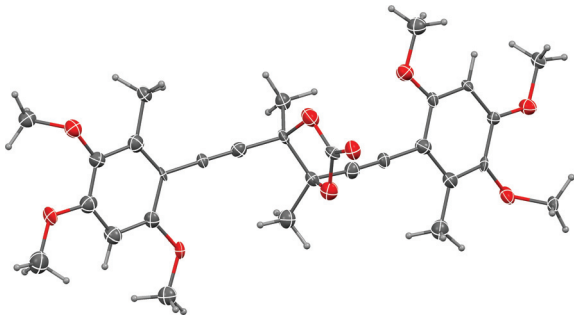


Fig. 3 Representation of the crystal structure of (±)-*epi*-antrodioxolanone (**51**). *R,R*-Enantiomer shown. Ellipsoids are shown at 50% probability amplitudes with hydrogen atoms assigned arbitrary radii.

coupling for aryl methyl ketones,[30] gave a complex mixture of products.

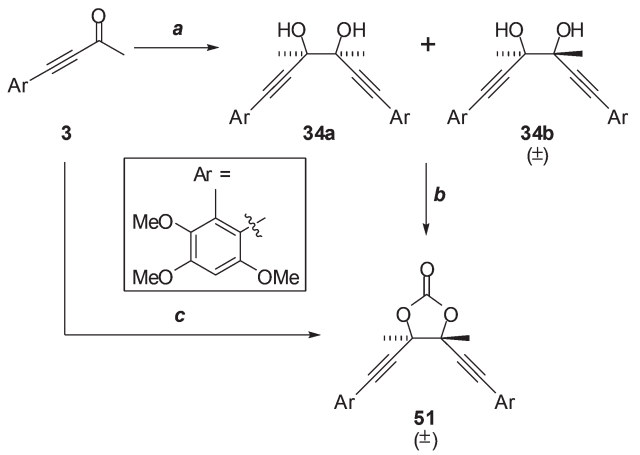
Of the reducing agents investigated, only SmI_2,[14,15] provided the pinacol coupling products **34a** and **34b**, in 55% yield, but unfortunately favouring the chiral isomers **34b** 10 : 1 (Scheme 13). The diastereomers were separable by HPLC but, disappointingly, and hampered by material availability, 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, (±)-*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 bis-tertiary glycol. A final attempt at the one-pot pinacol coupling/cyclisation using SmI_2 and methyl chloroformate[31] gave **51** directly, albeit in low yield, with none of the *meso* natural product **4** detected.

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 ± 3 μM, more effectively than ibuprofen (IC_{50} = 28 ± 3 μM).[1] Synthetic antrocamphin A 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 NFκB, a transcription factor that is a central player in the inflammatory cascade. Cytosolic NFκB is bound by the inhibitor IκB. Phosphorylation of IκB by the kinase IKK causes the NFκB–IκB complex to dissociate, allowing NFκB to enter the nucleus, where it induces transcription of a host of genes involved in the inflammatory response. Nuclear accumulation of NFκB was indeed dose-dependently decreased by antrocamphin A. Concurrently, expression of IκB 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 NFκB activation pathway, as a measure of anti-inflammatory activity.[32–34] To measure inhibition of NFκB pathway signalling, a Tumour Necrosis Factor (TNF) transcriptional reporter cell line was constructed by linking the green fluorescent protein (GFP) reporter gene to the NFκB-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



Scheme 13 Reagents, conditions and yields: (a) SmI_2, THF, 55% (1 : 10 mixture of **34a** : **34b**); (b) $CO(OCCl_3)_2$, pyridine, DCM, 16% (c) 1. SmI_2, THF; 2. $ClCO_2Me$ (32%).

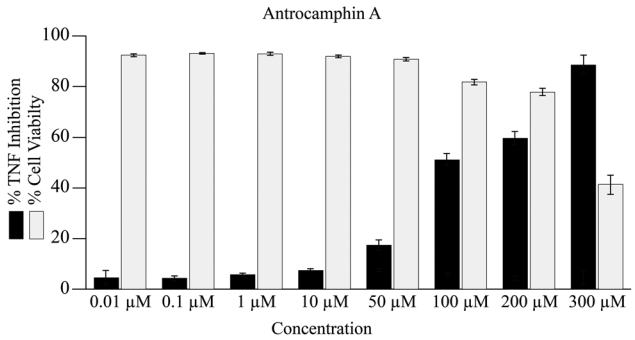


Fig. 4 Inhibition of TNF-reporter gene expression and consequence on cell viability following treatment with antrocamphin A (**2**) for 24 h. Data represent geometric mean expression levels of GFP driven by a TNF promoter, measured by flow cytometry (n = 3, bars represent mean ± SEM). Cell viability 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.

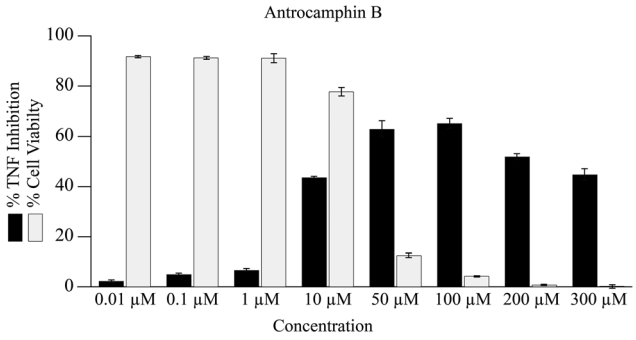


Fig. 5 Inhibition of TNF promoter transcriptional activity and consequence on cell viability following treatment with antrocamphin B (**3**) for 24 h. Data represent geometric mean expression levels of GFP driven by the TNF promoter measured by flow cytometry (n = 3, bars represent mean ± SEM). Cell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

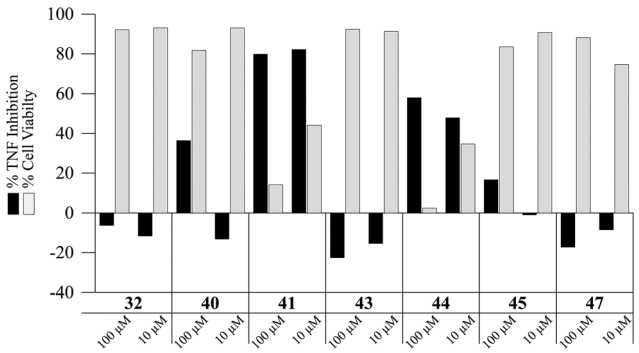


Fig. 6 Inhibition of TNF reporter gene expression and consequence on cell viability following treatment with synthetic intermediates and analogues of the antrocamphins and antrodioxolanone for 24 h. Data represent geometric mean expression levels of GFP driven by a TNF promoter measured by flow cytometry.

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 (Fig. 4). The results suggest an IC_{50} for NFκB-induced expression inhibition of approximately 100 μM; 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 μg 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 epithelioid carcinoma) – with ED_{50} values ≤10 μg mL^{-1}.[4]

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 NFκB 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 (Fig. 5, IC_{50} = 10.7 ± 0.3 μM [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 (Fig. 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.

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 hypothesis[3] that its anti-inflammatory effects arise, at least in part, by interfering with the nuclear localisation of the transcription factor

NFκB. 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 Kα 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]. ^{1}H NMR (400 MHz) δ 10.48 (s, 1H, CHO), 6.32 (s, 2H, 2 × ArH), 3.87 (s, 3H, OMe), 3.85 (s, 3H, OMe), 2.58 (s, 3H, Me). The ^{1}H NMR data are similar to those acquired at 300 MHz previously reported.[39]

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

30% Aqueous H_2O_2 (15.2 mL, 149 mmol) was added dropwise to a stirred solution of **7** (21.9 g, 122 mmol) and concentrated H_2SO_4 (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 H_2O (3 × 20 mL) and dried under vacuum to give **8a** white solid (19.8 g, 97%), m.p. = 103–104 °C [lit.[40] 103–104 °C]. ^{1}H 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 ^{1}H 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 K_2CO_3 (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 MHCl (200 mL). The aqueous phase was extracted with ether (6 × 80 mL). The extract was washed with saturated $NaHCO_3$ (50 mL), saturated NH_4Cl (50 mL) and water (50 mL), then dried and evaporated to give **10** as a colourless oil (2.96 g, 96%). ^{1}H 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 ^{1}H 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 μL, 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 × 60 mL). The extract was washed with H_2O (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. ^{1}H 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). ^{13}C NMR (125 MHz): δ 154.9 (ArO), 153.4 (ArO), 141.6 (ArO), 136.4 (Ar-Me), 95.0 (Ar-H), 82.3 (CI), 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 $C_{10}H_{13}IO_3^{\bullet+}$ requires: 307.9909. Microanalysis found: C 39.1, H 4.1%; calculated for $C_{10}H_{13}IO_3$: C 39.0, H 4.3%. The ^{1}H 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 H_2O (7 mL) was heated under reflux for 4 h, then cooled, diluted with H_2O (30 mL) and extracted with EtOAc (3 × 10 mL). The extract was washed with 10% $Na_2S_2O_4$ (2 × 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 H_2O (50 mL) and extracted with ether (4 × 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≡C). ^1H NMR (500 MHz, $CDCl_3$) 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, $J_{3\text{-Me},4}$ = 1 Hz, 3H, 3-Me); ^{13}C NMR (125.8 MHz; $CDCl_3$) 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 $C_{15}H_{18}O_3^{\bullet+}$ 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%), $Pd(PPh_3)_2Cl_2$ (77 mg, 1.5 mol%), DMSO (20 mL) and Et_2NH (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 H_2O (100 mL) and 1 M HCl (20 mL). The aqueous solution was extracted with EtOAc (3 × 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≡C); ^1H NMR (500 MHz, $CDCl_3$) 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); ^{13}C NMR (125.8 MHz; $CDCl_3$) 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 $C_{14}H_{18}O_4^{\bullet+}$ requires: 250.1205; Microanalysis found: C 66.4, H 7.1%; calculated for $C_{14}H_{18}O_4$ C 67.2, H 7.2%.

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

Method 1: activated MnO_2 (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, H5′), 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 (C2′), 101.4 (C1′), 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, $C_{14}H_{16}O_4$ 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 (≡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 $[M]^{\bullet+}$ (100), 103 (10); HRMS observed: 165.0551, $C_9H_9O_3^{\bullet+}$ 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), $Pd(PPh_3)_2Cl_2$ (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, H6′), 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 (C4′ or 1 or 2), 101.3 (C4′ or 1 or 2), 100.3 (C4′ or 1 or 2), 94.5 (C6′), 60.6 (MeO), 56.5 (MeO), 55.9 (MeO), 14.2 (3′-Me), 0.41 ($Si(Me)_3$); MS (EI) *m*/*z* 278 $[M]^{\bullet+}$, (100%), 263 (83), 248 (23), 233 (24); HRMS observed: 278.1345, $C_{15}H_{22}O_3Si$ 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%), $Pd(PPh_3)_2Cl_2$ (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 1 M 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, H5′), 3.87 (3H, s, OMe), 3.85 (3H, s, OMe), 3.71 (3H, s, OMe), 2.33 (3H, s, C2′-Me), 2.13 (1H, s, OH), 1.64 (6H, s, C2-Me). ^{13}C NMR (100 MHz) δ 157.6 (C4′ or C6′), 153.7 (C4′ or C6′), 141.4 (C3′), 135.7 (C2′), 104.6 (C1′), 101.1 (C3 & C4), 94.8 (C5′); 66.2 (C2), 60.7 (OMe), 56.6 (OMe), 56.1 (OMe), 32.0 (C1 & C2-Me), 14.3 (C2′-Me). MS (EI) *m*/*z*: 264.1 $[M]^{\bullet+}$ (18), 249.1 $[M - Me]^+$ (15), 246.1 $[M - H_2O]^{\bullet+}$ (100). HRMS (EI): observed, 264.1363. $C_{15}H_{20}O_4^{\bullet+}$ 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 1 M 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 (≡C–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, ≡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 (≡CH); 78.8 (Ar**C**≡), 60.4 (OMe), 56.2 (OMe), 55.8 (OMe), 14.0 (Me). MS (EI) *m*/*z*: 206 $[M]^{\bullet+}$ (100%), 191 $[M - Me]^+$ (99). HRMS (EI) observed: 206.0948, $C_{10}H_9O_3^{\bullet+}$ 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_2O was added dropwise to a stirred solution of **33** (0.21 g, 1.0 mmol) in Et_2O (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_2O (2 mL). The reaction mixture was heated for 24 h under reflux, then cooled, diluted with H_2O (50 mL) and extracted with EtOAc (3 × 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 °C. R_f (40% EtOAc–hexanes): 0.25; IR (thin film) ν_{max} cm^{-1}: 3100–3700 (OH), 2218 (C≡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 $[M]^{\bullet+}$ (20), 276 $[M - OH]^+$ (77), 233 (100). HRMS (EI): observed, 292.1312. $C_{16}H_{20}O_5^{\bullet+}$ 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_4Cl (20 mL) and H_2O (50 mL), then extracted with EtOAc (3 × 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 °C. R_f (40% EtOAc–hexanes): 0.25; IR (KBr) ν_{max} cm^{-1}: 2342 & 2140 (C≡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 $[M]^{\bullet+}$ (100), 395 $[M - Me]^+$ (22). HRMS (EI): observed, 410.1730. $C_{24}H_{26}O_6^{\bullet+}$ requires 410.1729.

4,5-Dimethyl-4,5-bis(phenylethynyl)-1,3-dioxolan-2-one (*cis*/*meso*40 and (±)-*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_2O (20 mL) and extracted with DCM (3 × 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 °C. R_f (20% EtOAc–hexanes): 0.35; IR (thin film) ν_{max} cm^{-1}: 2252 (C≡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≡ = C2′), 83.0 (C1′ or C4/5), 82.6 (C1′ or C4/5), 24.9 (Me). MS (EI) *m*/*z*: 272 $[M - CO_2]^{\bullet+}$ (1), 256 $[M - CO_3]^{\bullet+}$ (18), 128.0 (100). HRMS (ES): observed, 358.1440. $[C_{21}H_{17}O_3 + MeCN]^+$ 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≡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 $[M - CO_2]^{\bullet+}$ (8), 256 $[M - CO_3]^{\bullet+}$ (28), 128 (100). HRMS (ES): observed, 358.1441. $[C_{21}H_{17}O_3 + MeCN]^+$ 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%), $Pd(PPh_3)_2Cl_2$ (0.12 g, 1.5 mol%) and DMSO (24 mL) in a Young's flask at 50 °C was evacuated and backfilled with argon (×3). Et_2NH (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%), $Pd(PPh_3)_2Cl_2$ (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_2O (100 mL) and 1 M HCl (30 mL). The aqueous solution was extracted with EtOAc (4 × 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 °C. R_f (40% EtOAc–hexanes): 0.17; IR (thin film) ν_{max} cm^{-1}: 3000–3600 (OH), 2218 (C≡C). ^1H NMR (400 MHz): δ 6.33 (s, 1H, H5′), 4.56 (d, J = 5.6 Hz, 2H, H1), 3.88 (s, 3H, OMe), 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 $[M]^{\bullet+}$ (100), 221.0 $[M - Me]^+$ (84), 205 $[M - MeOH]^{\bullet+}$ (38). HRMS (EI): observed, 236.1044. $C_{13}H_{16}O_4^{\bullet+}$ 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 × 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: Ac_2O (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 H_2O (60 mL) and NEt_3 (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). ^{13}C 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 $[M]^{\bullet+}$ (100), 219.0 $[M - Me]^+$ (42). HRMS (EI): observed, 234.0898. $C_{13}H_{14}O_4^{\bullet+}$ requires 234.0892.

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

Freshly prepared Cu/Zn couple[45] (3.1 g, 1 : 1 –$CuSO_4 \cdot 5H_2O$: 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 H_2O (100 mL) and saturated $NaHCO_3$ (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). ^{13}C 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. $C_{26}H_{30}O_8^{\bullet+}$ 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 H_2O (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 $[M + H + H_2O]^+$ (50), 497 $[M + H]^+$

(100), 471 $[M + H - CO]^+$ (18), 453 $[M + H - CO_2]^+$ (89). HRMS (ES): observed 497.1821. $C_{27}H_{29}O_9^+$ requires 497.1806.

Isomer 1 (major): R_f (60% EtOAc–hexanes): 0.35. ^{1}H NMR $CDCl_3$ (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). ^{13}C NMR $CDCl_3$ (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; ^{1}H NMR $CDCl_3$ (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). ^{13}C NMR $CDCl_3$ (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-diyn-3,4-dione (47)

Ac_2O (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 H_2O (50 mL). The resulting precipitate was vacuum filtered to give an orange solid. The filtrate was diluted with 28% aqueous NH_3 (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). ^{1}H 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). ^{13}C 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. $C_{26}H_{27}O_8^+$ requires 467.1706.

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

Freshly prepared Cu/Zn couple[45] (3.01 g, 1 : 1 –$CuSO_4 \cdot 5H_2O$: 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). ^{1}H 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). ^{13}C 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. $C_{28}H_{39}O_8^+$ 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. ^{1}H 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 × 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 $[M + K - H_2]^+$ (20), 525 $[M + Na]^+$ (50), 523 $[M + Na - H_2]^+$ (35), 507 $[M + Na - H_2O]$ (10). HRMS (ES): observed 525.2468. $C_{28}H_{38}O_8Na^+$ 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 backfilling with argon after each addition. After 30 min of stirring the reaction mixture containing SmI_2 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 H_2O (20 mL) and the aqueous solution was extracted with EtOAc (3 × 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≡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 × 10 mm i.d., 5 μm, Apollo C18 reversed phase column (Grace-Davison) with a 33 mm × 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. ^{1}H NMR $CDCl_3$ (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 $CDCl_3$ (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. ^{1}H NMR $CDCl_3$ (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 $CDCl_3$ (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. $C_{28}H_{35}O_8^+$ requires 499.2326.

(±)-*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 H_2O (30 mL) and extracted with DCM (3 × 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≡C), 1787 (C═O). ^{1}H NMR $CDCl_3$ (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 $CDCl_3$ (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. $C_{29}H_{33}O_9^+$ 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 SmI_2. 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 H_2O (20 mL) and extracted with EtOAc (3 × 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]

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