The Synthesis of (*E*) and (*Z*)-Combretastatins A-4 and a Phenanthrene from *Combretum caffrum*

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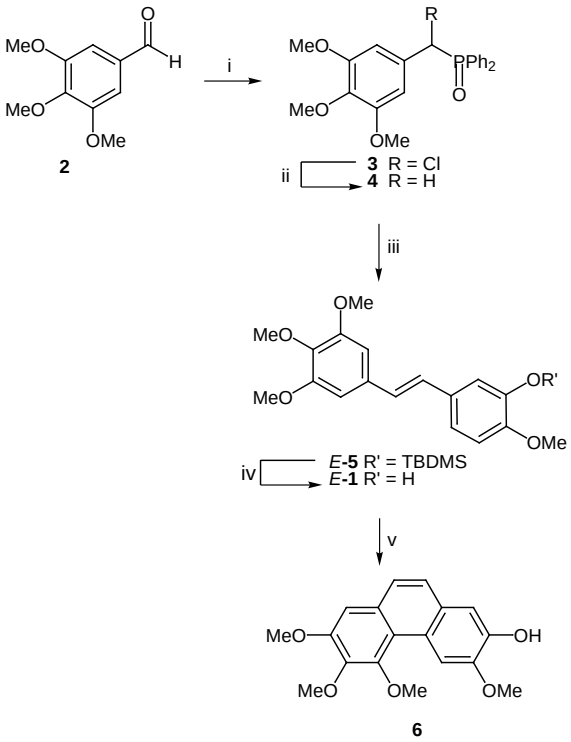
Abstract: The synthesis of the *trans* stilbene *E*-combretastatin A-4 has been achieved via a Horner–Wittig reaction of (3,4,5-trimethoxybenzyl)diphenylphosphine oxide. The anticancer drug *Z*-combretastatin A-4 was prepared by the hydroboration/protonation of a diaryl-alkyne.

Key words: alkynes, antitumor agents, alkenes, photochemistry, hydroboration

The stilbene *cis*-combretastatin A-4 *Z*-**1**, isolated from the African bush willow, *Combretum caffrum*[1] shows exciting potential as an anticancer agent binding strongly to tubulin[2] and displaying potent and selective toxicity[3] toward tumour vasculature.[4] *Cis*-combretastatin A-4 *Z*-**1** is able to inhibit cell growth at low concentrations (IC_{50}, P388 murine leukaemia cell line 2.6 nM). The potency of *trans*-combretastatin A-4 *E*-**1** is much lower and inhibits cell growth in the μM range. As part of a project devoted to the study of the anticancer effects of related compounds we required quantities of both geometrical isomers of combretastatin A-4. The efficient syntheses of both *cis*-combretastatin A-4 *Z*-**1** and its *trans* isomer *E*-**1** are described herein.

The route to the *trans* stilbene was based on the Horner–Wittig reaction of benzyldiphenylphosphine oxides which we had developed earlier.[5] α-Chlorobenzyldiphenylphosphine oxide (**3**) was prepared by a known reaction[6] between 3,4,5-trimethoxybenzaldehyde (**2**) and chlorodiphenylphosphine. The reduction of **3** to the (3,4,5-trimethoxybenzyl)diphenylphosphine oxide (**4**) was best achieved by catalytic hydrogenolysis thereby avoiding the use of tributyltin hydride,[7] which we had previously used to effect this transformation.[5] Phosphine oxide **3** was deprotonated with BuLi and reacted with *t*-butyldimethylsilyl protected isovanillin to give exclusively the *trans* alkene *E*-**5**. Removal of the silyl group, by treatment of **5** with TBAF gave *trans*-CA-4 *E*-**1** in excellent yield (96%). With *E*-**1** in hand we were able to achieve the total synthesis of a related natural product. Using the method of Katz and co-workers[8] oxidative photocyclisation[9] of *E*-**1** regiospecifically gave the phenanthrene **6**, a natural product isolated from *Combretum caffrum*[10] and *Combretum psidioides*.[11]



Reagents and conditions; i, Ph_2PCl, decalin reflux, 3 h, 63%; ii, Pd/C (10%), H_2 1 atm , MeOH:CH_2Cl_2 1:1, 2 days, 97%; iii, BuLi, 15 min, 0 °C; 4-MeO-3-OTBMDS-C_6H_3CHO, 12 h, 20 °C, 80%; iv, TBAF, 20 min, 20 °C, 96%; v hν, I_2, C_6H_6, r.t., 3 h, 68%.

Scheme 1

Our approach to the *cis* isomer is based on the synthesis of an alkyne **10** followed by selective hydrogenation. This is similar to an approach described recently by Furstner et al.;[12] however our protocol does not involve the use of protecting groups and the alkene moiety is constructed with greater stereoselectivity (*vide infra*).

(3,4,5-Trimethoxyphenyl)ethyne (**8**) was prepared by reaction of dibromoalkene **7** (from 3,4,5-trimethoxybenzaldehyde (**2**) and tetrabromomethane/triphenylphosphine) with BuLi. The other partner in the anticipated coupling reaction—5-iodo-2-methoxyphenol (**9**)—was made by the iodination of guaicol acetate with mercury(II) oxide

and iodine, using a modification of the procedure of Tassily and Leroide.[13] We found that the iodine was best added portionwise to a mixture of guaicol acetate and mercury(II) oxide. The alkyne **8** and aryl iodide **9** were then coupled according to our recently described method using tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide to give the diarylalkyne **10**.[14] The X-ray crystal structure of **10** (Figure 1) revealed that the two aromatic rings are orthogonal. The alkyne **10** was sequentially treated with dicyclohexylborane and acetic acid, according to the method of Zweifel et al.,[15] to give combretastatin A-4 *Z*-**1** in 82% yield. Inspection of the ^{1}H NMR spectrum of the crude reaction mixture indicated that the *Z*:*E* ratio was >99:1. However, purification by chromatography on silica resulted in partial isomerisation (the *Z*:*E* ratio of isolated *Z*-**1** was 95:5). Kugelrohr distillation of the crude reaction mixture proved to be the purification method of choice as the *Z*:*E* ratio remained >99:1.

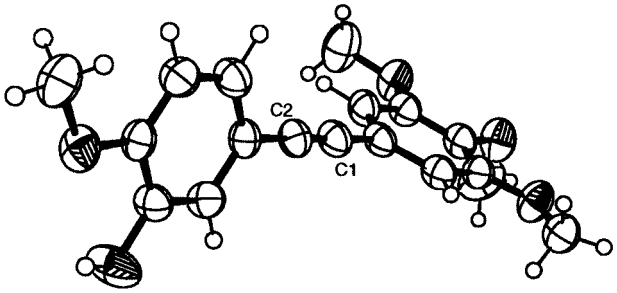
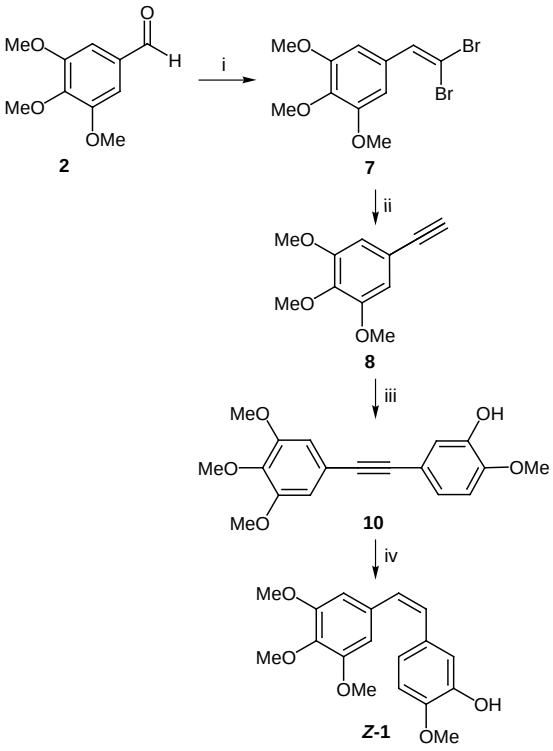


Figure 1 X-ray crystal structure of alkyne **10**

The growth inhibitory activities of *E*-**1** (IC_{50} 0.6 μM), phenanthrene **6** (IC_{50} 0.7 μM) and alkyne **10** (IC_{50} 21 μM) were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay. This assay is based on the reduction of yellow 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells to a purple-blue formazan, as detailed by Edmondson et al.[16] The IC_{50} concentration was calculated with reference to a standard curve constructed for control cells and represents the concentration which results in a 50% decrease in cell growth after five days incubation. The lower activity of the alkyne **10** is in agreement with our previous findings that diarylalkynes are not particularly cytotoxic. The phenanthrene **6** whilst moderately cytotoxic illustrates that the conformational flexibility present in *Z*-**1** is important in determining its high activity.

In conclusion we have developed efficient syntheses to both *cis* and *trans* combretastatin A-4, which will be useful in the design of analogues. The use of the hydroboration/protocol is a useful alternative to hydrogenation in the synthesis of *Z*-**1.**



Reagents and conditions; i Ph_3/CH_2Br_2, 0 °C, 10 min, 65%; ii, BuLi (2 equiv), -78 °C, 1 h then 20 °C, 1 h , 83%; iii, 5-iodo-2-methanoxyphenol (**9**), $(Ph_3P)_4Pd/CuI$, piperidine, 20 °C, 1 h, 63%; $(C_6H_{11})BH$, 0 °C, 1 h then 20 °C, 30 min; HOAc, 0 °C, 2 h 82%.

Scheme 2

The general experimental procedures used have been described elsewhere.[17]

(α-Chloro-3,4,5-trimethoxybenzyl)diphenylphosphine Oxide (3)

A mixture of **2** (28.06 g, 0.143 mol), chlorodiphenylphosphine (25.67 mL, 0.143 mol) and decalin (70 mL) was refluxed for 3 h, and allowed to cool. The decalin was decanted to yield a brown solid. The solid was recrystallised from propan-2-ol to furnish a white solid (37.27 g, 63%), mp 221–223 °C; R_f = 0.58 (SiO_2, EtOAc).

IR (KBr) ν = 3060 (w), 2940 (s), 1600–1430 (s), 1280 (m), 1240 (s), 1190 (s), 1020 (s), 720 (s), 580 (s) cm^{-1}.

^{1}H NMR (300 MHz; $CDCl_3$): δ = 3.67 (6H, s, 3,5-OMe), 3.81 (3H, s, 4-OMe), 5.36 (1H, d, J_{PH} = 4.7 Hz, α-H), 6.47 (2H, d, J_{PH} = 1.7 Hz, H-2 and H-6), 7.25-7.97 (10H, m).

FAB-MS *m/z* calcd for $C_{22}H_{22}ClO_4P$ (M^+) 416.0944, found 416.0949.

FAB-MS *m/z* (%) 416 (M, 40), 257 (100), 215 (80), 201 (50).

Anal calcd for $C_{22}H_{22}ClO_4P$,: C 63.4; H, 5.28; Cl, 8.51; P, 7.43; found: C, 63.6; H, 5.5; Cl, 8.7; P, 7.7.

(3,4,5-Trimethoxybenzyl)diphenylphosphine Oxide (4)

A slurry of 10% Pd on C (10 mol%) was stirred vigorously in anhyd MeOH (5 mL) under H_2 for 30 min. To this mixture a solution of **3** (0.83 g, 2 mmol) in MeOH (20 mL) and CH_2Cl_2 (20 mL) was added dropwise. The flask was charged with H_2 and the resulting mixture was stirred for 2 days. The mixture was filtered through Celite, dried ($MgSO_4$) and concentrated in vacuo to provide a white solid.

Recrystallisation (EtOAc) gave the **4** (0.74 g, 97%) mp 170–172 °C; R_f = 0.53 (SiO_2, EtOAc).

IR (KBr) ν = 3000–2700 (m), 1440 (s), 1250 (s), 1190 (s), 1000 (s) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.6 (2H, d, J_{PH} = 14.4 Hz, CH_2), 3.64 (6H, s, 3,5-OMe), 3.78 (3H, s, 4-OMe), 6.24 (2H, s, H-2, H-6), 7.45–7.54 (6H, m, aromatic), 7.71–7.73 (4H, m, aromatic).

Anal calcd for $C_{22}H_{23}O_4P$: C, 69.1; H, 6.1; P, 8.1; found C, 68.8; H, 5.9; P, 7.9.

FAB-MS m/z (%) 383 ([M+H]$^+$, 80), 181 (100).

(*E*)-1-(3',4',5'-Trimethoxyphenyl)-2-(4''-methoxy-3''-*tert*-butyldimethylsiloxyphenyl)ethene[(*E*)-5]

To compound **4** (1.70 g, 4.44 mmol) in anhyd THF (8 mL) was slowly added BuLi [2.8 mL, 4.44 mmol (1.6 M solution)] at 0 °C. The red solution was stirred for 15 min at 0 °C before 4-methoxy-3-(*tert*-butyl-dimethylsiloxy)benzaldehyde[15] (1.0 g, 3.70 mmol) in anhyd THF (10 mL) was added. The solution was stirred for an additional 30 min at 0 °C, allowed to slowly warm to r.t. and stirred overnight to afford a white precipitate of lithium diphenylphosphinate. The mixture was treated with H_2O (20 mL) and extracted with $CHCl_3$ (3 x 30 mL). The organic extracts were dried ($MgSO_4$) and evaporated in vacuo. Chromatography (silica gel, hexane:EtOAc 2:1 v/v) and recrystallisation (EtOH) gave white crystals (1.28 g, 80%), mp 127–128 °C (lit.[18] mp 128–130 °C); R_f = 0.75 (SiO_2, hexane:EtOAc 2:1 v/v).

IR (KBr) ν = 3060 (w), 2930 (m), 1590 (s), 1390 (w), 1350 (s), 1280 (s), 1130 (s), 1010-990 (m), 970 (s) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 0.19 (6H, s, $SiMe_3$), 1.02 (9H, s, CMe_3), 3.83 (3H, s, OMe), 3.86 (3H, s, OMe), 3.92 (6H, s, 2 x OMe), 6.71 (2H, s, H-2',6'), 6.83 (1H, d, *J* = 8.6 Hz, H-5''), 6.84 (1H, d, *J* = 17.3 Hz, CH=C), 6.92 (1H, d, *J* = 17.3 Hz, C=CH), 7.07–7.03 (2H, m, H-2,'' H-4'').

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = -4.6, 16.5, 25.8, 55.5, 56.1, 61.0, 103.3, 112.0, 118.6, 120.5, 126.6, 127.9, 130.4, 132.6, 135.4, 145.2, 150.9, 153.4.

Anal calcd for $C_{24}H_{34}O_5Si$ C, 66.9; H, 8.0; Si, 6.5; found C, 67.2; H, 7.9; Si, 6.5.

FAB-MS *m/z* (%) 430 ([M+H]$^+$, 100).

***trans*-Combretastatin-A4 [(*E*)-1].**

To alkene *E*-**5** (103 mg, 0.24 mmol) in anhyd THF, was added TBAF (2 mL of 0.5 M solution in THF, 1 mmol). The yellow solution was stirred for 20 min and then treated with H_2O (50 mL). The mixture was extracted with $CHCl_3$ (3 x 25 mL), and the organic extracts washed with H_2O (2 x 25 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (silica gel, EtOAc:hexane, 1:1 v/v) gave an off white solid (73 mg, 96%) mp 115–116 °C (lit.[18] mp 116 °C); R_f = 0.48 (SiO_2, EtOAc:hexane, 1:1 v/v).

IR (KBr) ν = 3440 (br), 960 (*trans*-HC=CH) cm^{-1}.

UV (MeCN): λ_{max} (ε) 222 (23 416), 330 nm (34 745).

^1H NMR (300 MHz; $CDCl_3$): δ = 3.68 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.92 (6H, s, OCH_3), 5.61 (1H, s, OH), 6.71 (2H, s, 2',6'-H), 6.83 (1H, d, *J* = 8.3 Hz, 5''-H), 6.87 (1H, d, *J* = 16.6 Hz, CH=C), 6.92 (1H, d, *J* = 16.6 Hz, C=CH), 6.98 (1H, dd, *J* = 8.3, 2.0 Hz, 6''-H), 7.14 (1H, d, *J* = 2.0 Hz, 2''-H).

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 55.9, 56.0, 60.9, 103.3, 111.6, 114.2, 119.1, 127.0, 127.7, 133.2, 137.6, 144.7, 145.7, 146.3, 153.3.

Anal calcd for $C_{18}H_{20}O_5$: C, 68.3; H, 6.4; found C, 68.9, H, 6.6.

FAB-MS *m/z* calcd for $C_{18}H_{20}O_5$: (M^+) 316.1311, found 316.1313.

FAB-MS *m/z* (%) 316 ([M+H]$^+$, 100).

2-Hydroxy-3,5,6,7-tetramethoxyphenanthrene (6)

Anhyd N_2 was passed through a solution containing stilbene *E*-**1** (0.05 g, 0.16 mmol), iodine (0.02 g, 0.16 mmol), propylene oxide (6.3 mL, 90 mmol) and benzene (500 mL) for 20 min. The solution was irradiated using a 125W UV lamp for approximately 3 h until the reaction was complete (TLC). The solution was washed with $Na_2S_2O_5$ (50 mL, aq, 15%), H_2O (50 mL) and brine (50 mL). The organic extract was dried ($MgSO_4$) and evaporated to give a yellow solid. Chromatography (silica gel, 2:1 hexane:EtOAc) gave an off-white solid (0.033 g, 68%), mp 175–177 °C (lit.[10] mp 176–178 °C); R_f = 0.69 (SiO_2, 2:1 hexane:EtOAc).

IR (KBr) ν = 3440 (br, OH), 3000–2740 (m), 1480–1460 (m), 1280 (s), 1270–1210 (s), 1170–1120 (d) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 4.01 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 5.88 (1H, s, OH), 7.08 (1H, s, H-1), 7.31 (1H, s, H-8), 7.51 (1H, d, *J* = 6.3 Hz, H-10), 7.53 (1H, d, *J* = 6.3 Hz, H-9), 9.07 (1H, s, H-4).

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 55.8, 55.9, 60.3, 61.3, 105.3, 107.1, 111.2, 118.7, 124.0, 124.9, 126.2, 127.7, 129.3, 142.5, 144.6, 146.7, 151.5, 151.7.

Anal calcd for $C_{18}H_{18}O_5$: C, 68.8; H, 5.7; found C, 68.5; H, 5.4.

FAB-MS *m/z* (%) 314 ([M+H]$^+$, 100).

5-Iodo-2-methoxyphenol[19] (9)

Guaicol acetate[20] (12 g, 72 mmol), Ac_2O (3.6 mL, 38 mmol) and HgO (12 g, 55 mmol) in CH_2Cl_2 (40 mL) were heated under reflux and I_2 (24 g, 95 mmol) was added portionwise slowly over 3 h. Each portion of iodine was added only after the orange colour of the reaction mixture reemerged. The resulting pink mixture was heated under reflux for further 12 h before being cooled and filtered. The solid residue obtained was washed thoroughly with H_2O. The filtrate was washed with $Na_2S_2O_5$ (aq, sat.) and H_2O (30 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The brown residue obtained was recrystallised (MeOH) to yield off-white crystals. These crystals were treated with NaOH (10 mL, aq 30% w/v) and heated under reflux. The boiling solution was acidified to pH 1 with concd HCl and allowed to cool. The precipitate which formed was filtered, washed with ice-cold water and recrystallised (MeOH) to give the phenol **9** as white crystals (14.2 g, 79%) mp 88–90 °C (lit.[21] mp 87–88 °C); R_f = 0.81 (SiO_2, EtOAc:hexane 1:2).

IR (KBr) ν = 3500–3400 (br), 3000–2740 (m), 1300 (s), 1220 (s), 1130 (m) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.86 (3H, s, OMe), 5.61 (1H, s, OH), 6.59 (1H, d, *J* = 8.5 Hz, H-3), 7.16 (1H, dd, *J* = 8.5, 2.1 Hz, H-4), 7.24 (1H, d, *J* = 2.1 Hz, H-6).

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 56.1, 83.1, 112.6, 123.5, 129.1, 146.6, 146.8.

Anal calcd for $C_7H_7O_2I$: C, 33.6; H, 2.8; found C, 33.7; H, 2.7.

FAB-MS *m/z* (%) 250 ([M+H]$^+$, 100).

1,1-Dibromo-2-(3',4',5'-trimethoxyphenyl)ethene[22] (7)

Triphenylphosphine (3.16 g, 12.06 mmol) was added to CBr_4 (2 g, 6.03 mmol) in anhyd CH_2Cl_2 (45 mL) at 0 °C. Compund **2** (1.18 g, 6.03 mmol) was added and the resulting mixture was stirred at 0 °C for 10 min before the addition of H_2O (15 mL). The organic phase was separated, dried ($MgSO_4$) and concentrated in vacuo. The yellow slurry obtained was tritrated with hexane and then Et_2O to remove Ph_3PO. The organic washings were dried ($MgSO_4$) and concentrated in vacuo to give the dibromoalkene (2.12 g, 65%) as a

yellow solid, mp 43–45 °C (lit.[22] mp 40 °C); R_f = 0.74 (SiO_2, hexane:EtOAc, 3:1).

IR (KBr) ν = 3000–2700 (m), 1130 (m) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.86 (6H, s, 2 x OMe), 3.87 (3H, s, OMe), 6.80 (2H, s, H-2',6'), 7.41 (1H, s, H-1).

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 56.2, 60.8, 105.8, 130.5, 136.6, 138.4, 153.0, 205.7.

Anal calcd for $C_{11}H_{12}Br_2O_3$: C, 37.5; H, 3.4; Br, 45.4; found C, 37.7; H, 3.3; Br, 45.2.

FAB-MS *m/z* (%) 352 ([M+H]$^+$, 100).

3,4,5-Trimethoxyphenylethyne (8)

BuLi [(3.75 mL, 6 mmol) of a 1.6 M solution] was added slowly (over 1 h) to the dibromoalkene **7** (1 g, 2.9 mmol) in anhyd THF (20 mL) cooled to -78 °C. The resulting brown solution was stirred at -78 °C for 1 h then warmed slowly to r.t. and stirred for a further h. Sat. NH_4Cl (50 mL) was added and the mixture extracted with ether (3 x 50 mL). The combined etheral extracts were dried ($MgSO_4$) and concentrated in vacuo to afford a yellow solid. Chromatography (silica gel, CH_2Cl_2) gave the alkyne **7** (0.46 g, 83%) as a white solid, mp 68–69 °C (lit.[23] mp 68–68.5 °C); R_f 0.50 (SiO_2, 4:3 hexane:EtOAc).

IR (KBr) ν = 3250 (s), 3000–2700 (Ar) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.03 (1H, s, CH), 3.85 (9H, s, 3 x OCH_3), 6.73 (2H, s, H-2,6).

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 56.0, 60.8, 76.1, 83.6, 109.2, 116.9, 139.2, 153.9.

Anal calcd for $C_{11}H_{12}O_3$: C, 68.7; H, 6.3; found C, 69.1; H, 6.6.

FAB-MS *m/z* (%) 193 ([M+H]$^+$, 25).

2-(3"-Hydroxy-4"-methoxyphenyl)-1-(3',4',5'-trimethoxyphenyl)ethyne[12] (10)

Under N_2 alkyne **8** (0.5 g, 2.6 mmol), phenol **9** (0.65 g, 2.6 mmol), $(PPh_3)_4Pd$ (0.14 g, 0.52 mmol) and CuI (0.02 g, 0.13 mmol) were dissolved in piperidine (20 mL) to give a clear green solution. The solution was stirred for 1 h at r.t. during which time a yellow precipitate slowly formed. The precipitate dissolved upon addition of sat. NH_4Cl (20 mL). The mixture was extracted with Et_2O (3 x 20 mL). The organic extracts were washed with H_2O (2 x 20 mL) and brine (2 x 20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (silsca gel, hexane:EtOAc 4:3) and recrystallisation (hexane:$CHCl_3$, 1:1) gave the diarylalkyne **10** (0.51 g, 63%) as white crystals, mp 96–98 °C; R_f = 0.87 (SiO_2, EtOAc:hexane, 1:2 v/v).

IR (KBr) ν = 3540–3300 (br), 3000–2940 (s), 2220 (w), 1580 (s), 1240 (s), 1130 (s) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.86 (3H, s, OCH_3), 3.88 (6H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.62 (1H, s, OH), 6.75 (2H, s, H-2',6'), 6.82 (1H, d, *J* = 8.3 Hz, H-5"), 7.06 (1H, dd, *J* = 8.3 and 1.9 Hz, H-6"), 7.09 (1H, d, *J* = 1.9 Hz, H-2").

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 55.9, 56.2, 61.0, 86.9, 87.9, 108.7, 110.5, 116.0, 117.5, 118.6, 124.2, 133.6, 145.4, 147.1, 153.1.

Anal calcd for $C_{18}H_{18}O_5$: C, 68.8; H, 5.8; found C, 68.8; H, 5.8.

FAB-MS *m/z* (%) 314 ([M+H]$^+$, 100).

Crystal data for **10**: $C_{18}H_{18}O_5$, *M* = 314.32, triclinic, *a* = 8.622(2), *b* = 9.196(2), *c* = 10.762(3) Å, *U* = 816.5(3) Å^3, *T* = 293(2) K, space group P -1, monochromated Mo-Kα radiation, λ = 0.71073 Å, *Z* = 2, *Dc* = 1.279 Mg m^{-3}, *F*(000) = 332, colourless plates, dimensions 0.35 x 0.35 x 0.30 mm, μ(Mo–Kα) = 0.193 mm^{-1}, Siemens R3M/N diffractometer, ω-2θ scan, 4<2θ<50°, 3479 reflections measured, 2870 unique reflections. The structure was solved by direct methods and refined by full-matrix least-squares (SHELX 97). All non-hydrogen atoms were refined anisotropically; hydrogens were constrained to chemically-reasonable positions. The final cycle of least-squares refinement (for 280 parameters) converged with *wR*2 = 0.1268 (for all data) and *R*1 = 0.0431 (for 2870 reflections [I>2σ(I)]). Selected bond distances: C(1)-C(2) 1.186(2) Å.

***cis*-Combretastatin-A4 *Z*-1**

Borane·THF (5 mL of a 1 M solution in THF) was slowly added to distilled cyclohexene (1.02 mL, 10 mmol) in THF (5 mL) at 0 °C. This mixture was stirred for 1 h at 0 °C, affording a dense white precipitate after 10 min. The alkyne **10** (0.75 g, 2.4 mmol) in THF (5 mL) was added and the resulting yellow mixture was stirred for 1 h at 0 °C after which time the precipitate had dissolved giving a clear yellow solution. The solution was allowed to warm to r.t. and stirred for 30 min. HOAc (1.72 mL, 30 mmol) was added and the solution stirred at 0 °C for 2 h. H_2O (30 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 25 mL). The organic extracts were washed with sat. $NaHCO_3$ (3 x 25 mL), H_2O (30 mL), dried ($MgSO_4$) and concentrated in vacuo. Impurities were removed by Kugelrohr distillation leaving the alkene *Z*-**1**[1] (0.62 g, 82%) as the residue; R_f = 0.73 (SiO_2, EtOAc:hexane, 1:1 v/v).

IR (KBr) ν = 3460-3400 (br), 3000 (m), 1620 (m), 1580 (s), 1240 (s), 1030–1010 (m) cm^{-1}.

^1H NMR (300 MHz; $CDCl_3$): δ = 3.70 (6H, s, OMe), 3.84 (3H, s, OMe), 3.86 (3H, s, OMe), 5.52 (1H, s, OH), 6.41 (1H, d, *J* = 12.2 Hz, CH=C), 6.47 (1H, d, *J* = 12.2 Hz, C=CH), 6.52 (2H, s, H-2', 6'), 6.73 (1H, d, *J* = 8.4 Hz, H-5"), 6.80 (1H, dd, *J* = 8.4, 1.9 Hz, H-6"), 6.92 (1H, d, *J* = 1.9 Hz, H-2").

^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 55.9, 60.9, 106.0, 110.3, 115.0, 121.1, 129.0, 129.5, 130.6, 132.9, 137.1, 145.2, 145.7, 152.8.

Anal calcd for $C_{18}H_{20}O_5$: C, 68.4; H, 6.4; found C, 68.0; H, 6.4.

FAB-MS *m/z* (%) 316 ([M+H]$^+$ 100).

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References and Notes

(1) Pettit, G. R., Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
(2) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984.
(3) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. *Brit. J. Cancer* **1995**, *71*, 705.
(4) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Research* **1997**, *57*, 1829.
(5) Brown, K. M.; Lawrence, N. J.; Liddle, J.; Muhammad, F.; Jackson, D. A. *Tetrahedron Lett.* **1994**, *35*, 6733.
(6) De' ath, N. J.; Miller, J. A.; Nunn, M. J.; Stewart, D. *J. Chem. Soc., Perkin Trans. 1* **1981**, 776.
(7) With AIBN and tributyltinhydride the yield of **3** → **4** was 66%.
(8) Liu, L.; Yang, B.; Katz, T. J.; Poindexter, M. K. *J. Org. Chem.* **1991**, *56*, 3769.
(9) Mallory, F. B.; Mallory, C. W. *Org. React.* **1984**, *30*, 1.
(10) Pettit, G. R.; Singh, S.B.; Niven, M. L.; Schmidt, J. M. *Can. J. Chem.* **1988**, *66*, 406.

(11) Letcher, R. M.; Nhamo, L. R. M. *J. Chem Soc., Perkin Trans 1* **1972**, 2941.
(12) Furstner, A.; Nikolakis, K. *Liebigs Ann. Chem.* **1996**, 2107.
(13) Tassily, E.; Leroide, J. *Bull. Soc, Chim. Fr.* **1907**, *1*, 930.
(14) Hadfield, J. A.; McGown, A. T. *Synth. Commun.* **1998**, *28*, 1421.
(15) Brown, H. C.; Zweifel, G. *J. Am. Chem. Soc.* **1961**, *83*, 3834.
(16) Edmondson, J. M.; Armstrong, L. S.; Martinez, A. O. *J. Tissue Culture Methods* **1988**, *11*, 15.
(17) Bushell, S. M.; Crump, J. P.; Lawrence, N. J.; Pineau, G. *Tetrahedron* **1998**, *54*, 2269.
(18) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666.
(19) Boden, N.; Bushby, R. J.; Cammidge, A. N. *J. Am. Chem. Soc.* **1995**, *117*, 926**.**
(20) From guaicol and acetyl chloride in CH_2Cl_2; Mori, M.; Ishikura, M.; Ikeda, T.; Ban, Y. *Heterocycles* **1984**, *22*, 253.
(21) Feldman, K. S. *J. Org. Chem.* **1997**, *62*, 4983.
(22) Bestmann, H. J.; Frey, H. *Liebigs Ann. Chem.* **1980**, 2061.
(23) Kato, M.; Kido, F.; Wu, M.-D.; Yoshikoshi, A. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1516.

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