The Synthesis of a Combretastatin A-4 Based Library and Discovery of New Cooperative *ortho*-Effects in Wittig Reactions Leading to (*Z*)-Stilbenes

David C. Harrowven,*[a] Ian L. Guy,[a] Melanie Howell,[b] Graham Packham[b]

[a] School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK
Fax +44(23)80596805; E-mail: dch2@soton.ac.uk

[b] Cancer Research UK Clinical Centre, University of Southampton School of Medicine, Southampton, SO16 6YD, UK
E-mail: G.K.Packham@soton.ac.uk

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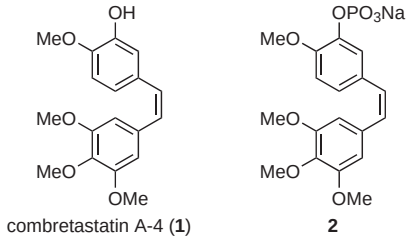
Abstract: A synthesis of combretastatin A-4 and a small library of analogues led to the discovery of some new cooperative *ortho*-effects allowing (*Z*)-stilbenes to be prepared in high yield and diastereomeric ratio.

Key words: alkenes, antitumor agents, stereoselective synthesis, total synthesis, Wittig reactions

Though structurally simple, combretastatin A-4, from the african bush willow *Combretum caffrum* (Combretaceae), has been shown to be a potent inhibitor of cancer cell growth and a competitive inhibitor of colchicine binding to tubulin.[1] It is a potent inhibitor of mitosis and microtubule assembly and is capable of inducing irreversible vascular shutdown within solid tumours while leaving normal vasculature intact.[1] Poor bioavailability for combretastatin A-4 led to the development of sodium phosphate analogue **2**, which advanced to Phase I human cancer clinical trials in 1998 and is currently undergoing evaluation in human cancer Phase II and combination trials (Figure 1).[2,3]



combretastatin A-4 (**1**) **2**

Figure 1

The chemical synthesis of combretastatin A-4 has been tackled in a variety of ways.[3] Strategies based on the use of olefination reactions to form the stilbene tend to be shorter than those designed to control alkene geometry from the outset. However, they generally suffer from poor stereocontrol, which necessitates a troublesome separation of combretastatin A-4 from its isomer (*E*)-**1**. Herein we describe a new approach to combretastatin A-4 and a small library of analogues that exploits cooperative *ortho*-effects in Wittig reactions to achieve high *Z*-selectivity in the olefination reaction.[4]

The idea stemmed from a report by Gilheany et al. who showed that Wittig reactions in which the ylide and arylaldehyde each had a single *ortho*-fluoro-, chloro- or bromo-substituent proceeded with a *Z*:*E* selectivity of >6:1.[4] We reasoned that these 'co-operative *ortho*-effects' could be exploited to give stilbene (*Z*)-**8a** from aldehyde **7a** and phosphonium salt **5** in high yield and with high dr. Reductive removal of the aryl halides would then give combretastatin A-4 (**1**), to complete a short stereoselective synthesis.

To that end, aldehyde **7a** was prepared by bromination of isovanillin (**6**) using bromine in acetic acid.[5] Contemporaneously, treatment of 3,4,5-trimethoxybenzyl alcohol (**3**) with bromine in acetic acid then triphenylphosphine gave phosphonium salt **5** (Scheme 1).[4c] Exposure of **5** to potassium *tert*-butoxide next facilitated condensation with aldehyde **7a** leading to a 9:1 mixture of (*Z*)- and (*E*)-stilbenes (**8a**). Finally, adding 3.5 equivalents BuLi to stilbenes **8a** gave, after a water quench, a 9:1 mixture of combretastatin A-4 (**1**) and (*E*)-**1**, which were separated by column chromatography on silica gel.

In seeking a further enhancement of *Z*-selectivity, we next applied the sequence to *o*-iodoarylaldehydes **7b**–**d** (Scheme 1).[6] The Wittig reactions between phosphonium salt **5** and aldehydes **7b** and **7c** showed no improvement in dr compared to the aforementioned condensation with **7a**. However, union of **7d** and **5** gave (*Z*)-**8d** in 80% yield after column chromatography, essentially free of the traces of (*E*)-**8d** (5%) that were formed as a by-product (dr = ca. 15:1, as indicated by isolated masses and ^{1}H NMR analysis of the crude product mixture prior to column chromatography). The allyl protecting group was smoothly removed using 5 mol% $Pd(PPh_3)_4$ and morpholine in THF at room temperature to give (*Z*)-**8b** with no discernible loss of stereochemical integrity.[7] Halogen–lithium exchange with BuLi, followed by protonation, completed the synthesis of combretastatin A-4 (*Z*)-**1**.

The high *Z*-selectivities achieved using **7b**–**d** were notable from a chemical perspective as they demonstrated for the first time that the cooperative *ortho*-effect extended to *ortho*-iodoarylaldehydes. In the second phase of the

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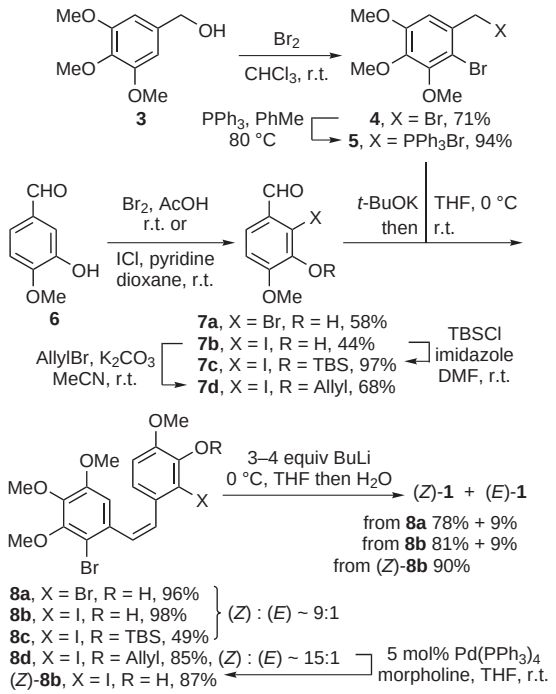
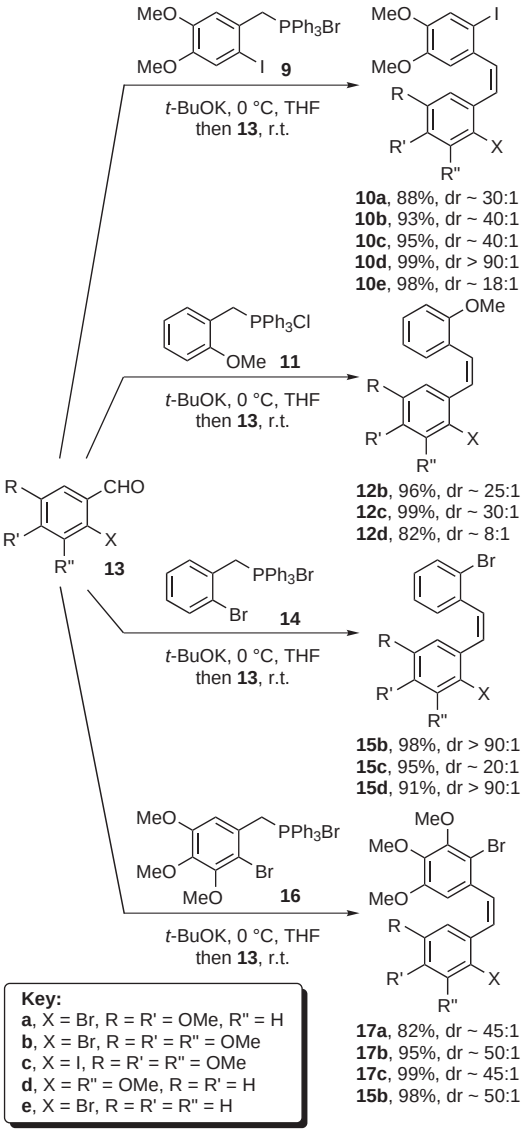


Scheme 1 Short syntheses of combretastatin A-4.

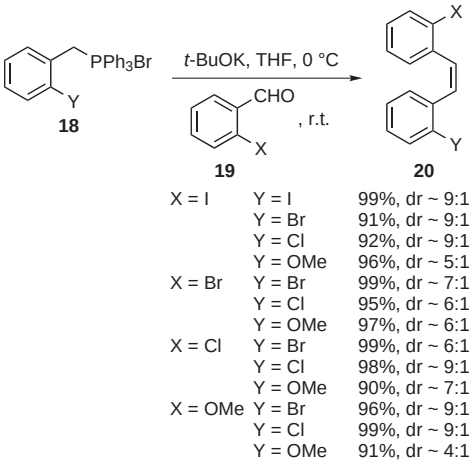
project aimed at analogue synthesis, we went on to show that the effect extended to *ortho*-iodo- and *ortho*-alkoxy substituents in both the ylide and aromatic aldehyde.[8] Thus, Wittig reactions between *ortho*-iodobenzyl phosphonium salt **9** and arylaldehydes **13a**–**e** gave stilbenes **10a**–**e**, respectively, in high yield and with remarkable *Z*-selectivity, dr ≥ 18:1 (Scheme 2).

Z-Selectivities substantially greater than expected from literature precedent[4] were also observed in Wittig reactions between (2-methoxybenzyl)triphenylphosphonium chloride (**11**) and arylaldehydes **13b**–**d**, suggesting that electron-donating substituents on the arenes also influence the stereochemical course of the reaction. To confirm that hypothesis, a series of coupling reactions between the ylides derived from **14**, **16** and **18** (X = I, Br, Cl, OMe), and aldehydes **13a**–**e** and **19** (X = I, Br, Cl, OMe), was conducted. The results attained show that Wittig reactions leading to simple *o,o′*-disubstituted stilbenes (Scheme 3) generally give lower *Z*-selectivities than comparable reactions leading to electron-rich stilbenes (Scheme 2).

Having succeeded in generating an array of combretastatin A-4 analogues in high chemical yield and with excellent stereocontrol, our next task was to screen the series for biological activity. Initial assessment of growth inhibitory activity in ZR-75-1 breast cancer cells demonstrated that combretastatin A-4 (**1**) and its dibromide **8a** were active, whereas stilbenes **10a**, **10b**, **12b**, **12d**, **15b**, **15d**, **17a**, **17b**, **17c**, and phenanthrenes **21**–**26** (Figure 2) gave less than 30% inhibition at 1 mM and were therefore considered inactive. Dose response assays demonstrated that combretastatin A-4 (**1**) and dibromide **8a** inhibited ZR-75-1 cell growth with EC_{50} values of 3.5 ± 2.3 and



Scheme 2 The use of cooperative *ortho*-effects to achieve *Z*-selective Wittig reactions leading to stilbenes.



Scheme 3 The use of cooperative *ortho*-effects to achieve *Z*-selective Wittig reactions leading to *o,o′*-disubstituted stilbenes.

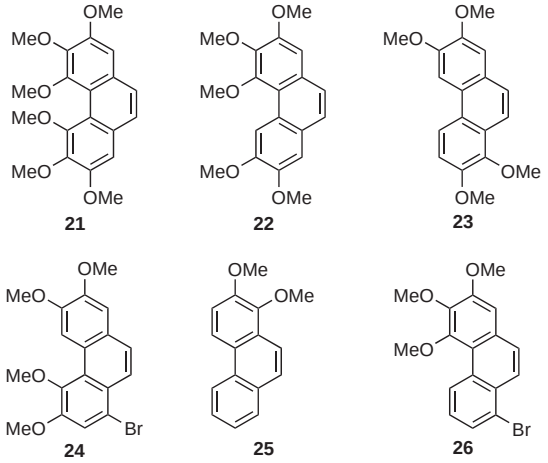


Figure 2 Phenanthrenes assessed for growth inhibitory activity in ZR-75-1 breast cancer cells.

51 ± 4.0 nM, respectively (mean of two independent experiments ± sd). To confirm that growth inhibition was due to inhibition of microtubule polymerization, we measured the effects of the compounds on progression through the cell cycle and on phosphorylation of the Bcl-2 protein, hallmark responses of cells treated with anti-tubulin agents.[9] Like combretastatin A-4 (**1**), its dibromide **8a** induced cell cycle arrest in the G2M phase of the cell cycle and increased Bcl-2 phosphorylation, consistent with acting as anti-tubulin agents (Figure 3).

In summary, a short and efficient route to combretastatin A-4 has been developed and used in the preparation of a small library of analogues. Of these, dibromocombretastatin A-4 (**8a**) was identified as a potent new cell-growth inhibitor. From a chemical perspective, the traceless use of aryl halides to control the stereochemical course of Wittig reactions leading to (*Z*)-stilbenes is notable, as is our demonstration cooperative *ortho*-effects extend to iodo and methoxy substituents in both the aldehyde and ylide components, and are further enhanced by additional electron donating substituents.[10]

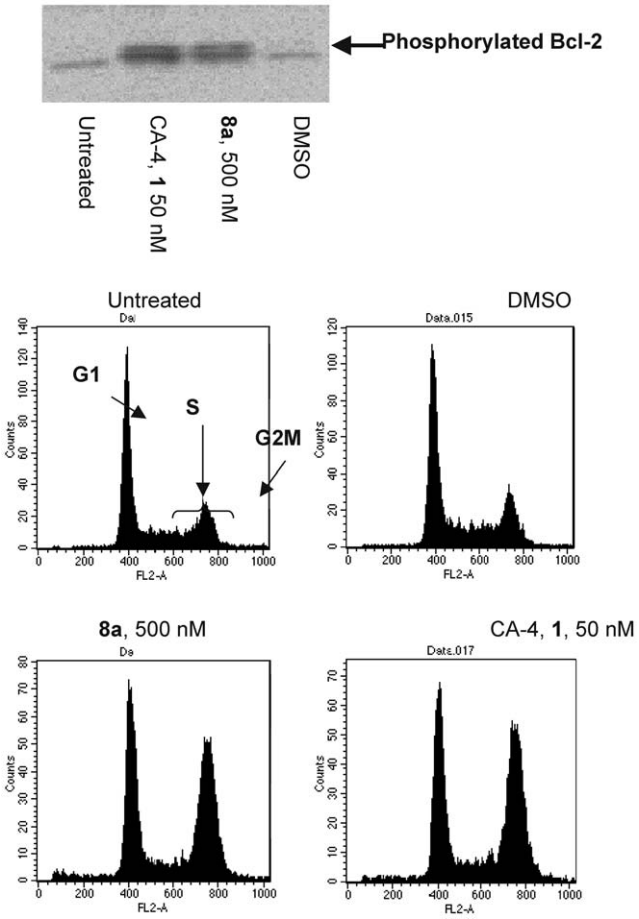


Figure 3 Effect of compounds on Bcl-2 phosphorylation and cell cycle arrest. ZR-75-1 cells were treated with the indicated concentrations of combretastatin A4 (**1**) or compound **8a** (at approx. $10 \times IC_{50}$), DMSO as a solvent control or left untreated (UT). Cells were collected after 24 h and analysed for (*A*) Bcl-2 phosphorylation and (*B*) cell cycle distribution by Western blotting and flow cytometry, respectively. In (*A*), the position of phospho-Bcl-2 is indicated. In (*B*), the different phases of the cell cycle are indicated on the first histogram.

(2-Bromo-3,4,5-trimethoxybenzyl)triphenylphosphonium Bromide (5)

A solution of dibromide **4** (14.51 g, 42.7 mmol) and PPh_3 (11.19 g, 42.7 mmol) in toluene (150 mL) was heated at 80 °C (150 mL) for 16 h then cooled to r.t. The resulting precipitate was collected by filtration, washed with toluene (50 mL) and dried in vacuo to give **5** (24.23 g, 40.3 mmol, 94%) as a white solid, mp 210–213 °C (EtOH). IR (neat): ν_{max} = 3021 (w), 2970 (w), 2945 (w), 2868 (w), 2844 (w), 1588 (w), 1570 (w), 1483 (m), 1462 (m), 1435 (s), 1393 (m), 1341 (s), 1250 (m), 1099 (vs) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.77 (3 H, td, *J* = 7.1, 2.0 Hz), 7.71–7.57 (12 H, m), 7.06 (1 H, d, *J* = 2.8 Hz), 5.50 (2 H, d, *J* = 13.7 Hz), 3.80 (3 H, s), 3.67 (3 H, s), 3.54 (3 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.0 (d, *J* = 4.0 Hz, C), 151.0 (d, *J* = 3.0 Hz, C), 143.5 (d, *J* = 5.0 Hz, C), 135.3 (d, *J* = 3.0 Hz, 3 × CH), 134.5 (d, *J* = 9.9 Hz, 6 × CH), 130.3 (d, *J* = 12.9 Hz, 6 × CH), 122.4 (d, *J* = 8.9 Hz, C), 117.4 (d, *J* = 86.2 Hz, 3 × C), 113.8 (d, *J* = 6.9 Hz, C), 112.5 (d, *J* = 5.0 Hz, CH), 61.3 (CH_3), 60.9 (CH_3), 56.5 (CH_3), 31.2 (d, *J* = 47.6 Hz, CH_2). MS (ES$^+$): *m/z* (%) = 523 (100) [M(^{81}Br) – Br]$^+$, 521 (98) [M(^{79}Br) – Br]$^+$. Anal. Calcd for $C_{28}H_{27}Br_2O_3P$ (%): C, 55.84; H, 4.52. Found: C, 55.88; H, 4.51.

(*Z*)-1-[2-(3-Allyloxy-2-iodo-4-methoxyphenyl)-1-ethenyl]-2-bromo-3,4,5-trimethoxybenzene (8d)

To a cooled (0 °C) suspension of phosphonium salt **5** (542 mg, 0.9 mmol) in THF (10 mL) was added *t*-BuOK (118 mg, 1.05 mmol). After 30 min, 3-allyloxy-2-iodo-4-methoxybenzaldehyde (**7d**, 239 mg, 0.75 mmol) in THF (10 mL) was added over 10 min. The reaction was allowed to warm to r.t. and after 16 h H_2O (20 mL) was added. The aqueous phase was separated and extracted with EtOAc (3 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), concentrated in vacuo and purified by column chromatography (SiO_2, 20% EtOAc–PE) to yield firstly a 4:1 mixture of (*Z*)-**8d** and (*E*)-**8d** (92 mg, 0.16 mmol, 21%) as a colorless oil, followed by a 50:1 mixture of (*Z*)-**8d** and (*E*)-**8d** (269 mg, 0.48 mmol, 64%) as a colorless oil. The early fractions were re-purified by column chromatography (SiO_2, 20% EtOAc–PE) giving further (*Z*)-**8d** (70 mg, 0.12 mmol, 15%) and (*E*)-**8d** (18 mg, 0.04 mmol, 4%).

Compound (*Z*)-**8d**: IR (neat): ν_{max} = 2999 (w), 2935 (w), 2838 (w), 1583 (w), 1556 (w), 1475 (s), 1387 (s), 1325 (m), 1289 (m), 1243 (m), 1159 (m), 1103 (s), 1006 (s), 923 (m) cm^{-1}. ^1H NMR (300

MHz, $CDCl_3$): δ = 6.75 (1 H, d, *J* = 8.4 Hz), 6.69 (1 H, d, *J* = 8.4 Hz), 6.66 (1 H, d, *J* = 11.7 Hz), 6.61 (1 H, d, *J* = 11.7 Hz), 6.32 (1 H, s), 6.19 (1 H, ddt, *J* = 17.1, 10.3, 6.0 Hz), 5.42 (1 H, dq, *J* = 17.1, 0.5 Hz), 5.25 (1 H, dd, *J* = 10.3, 1.5 Hz), 4.53 (2 H, d, *J* = 6.0 Hz), 3.90 (3 H, s), 3.85 (3 H, s), 3.81 (3 H, s), 3.40 (3 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.1 (C), 151.4 (C), 150.9 (C), 147.8 (C), 142.3 (C), 134.8 (CH), 134.6 (C), 134.0 (CH), 132.5 (C), 129.8 (CH), 126.1 (CH), 118.3 (CH_2), 112.5 (CH), 110.7 (C), 109.8 (CH), 98.9 (C), 74.0 (CH_2), 61.3 (CH_3), 61.1 (CH_3), 56.2 (CH_3), 55.8 (CH_3). MS (ES^+): *m/z* (%) = 585 (100) [M(^{81}Br) + Na]$^+$, 583 (88) [M(^{79}Br) + Na]$^+$.

(*Z*)-1-[2-(3-Hydroxy-2-iodo-4-methoxyphenyl)-1-ethenyl]-2-bromo-3,4,5-trimethoxybenzene (8b)

To a solution of stilbene (*Z*)-**8d** (102 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (10 mg, 5 mol%) at r.t. was added morpholine (0.32 mL, 3.64 mmol) slowly and the reaction was stirred at r.t. for 48 h. The reaction mixture was concentrated in vacuo and the residue was redissolved in Et_2O (15 mL). The organic phase was washed with 2 M HCl (3 × 10 mL), dried ($MgSO_4$), concentrated in vacuo and purified by column chromatography (SiO_2, 20% EtOAc–PE) to yield (*Z*)-**8b** (90 mg, 0.17 mmol, 95%) as a colorless oil. IR (neat): ν_{max} = 3550–3200 (br w), 3001 (w), 2937 (w), 2840 (w), 1595 (w), 1557 (w), 1479 s, 1388 (m), 1325 (m), 1272 (m), 1239 (m), 1197 (m), 1165 (m), 1102 (s), 1027 (s), 1006 (s), 923 (m) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.68 (1 H, d, *J* = 11.8 Hz), 6.67–6.61 (2 H, m), 6.62 (1 H, d, *J* = 11.8 Hz), 6.35 (1 H, s), 6.17 (1 H, s, OH), 3.90 (3 H, s), 3.85 (6 H, s), 3.42 (3 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.1 (C), 150.9 (C), 145.8 (C), 144.8 (C), 142.4 (C), 134.6 (CH), 134.4 (C), 132.6 (C), 130.0 (CH), 121.9 (CH), 110.7 (C), 110.5 (CH), 109.8 (CH), 87.2 (C), 61.3 (CH_3), 61.1 (CH_3), 56.5 (CH_3), 55.9 (CH_3). MS (EI): *m/z* (%) = 522 (9) [M(^{81}Br)]$^+$, 520 (8) [M(^{79}Br)]$^+$, 314 (26), 207 (65), 154 (61), 44 (100). MS: *m/z* calcd for $C_{18}H_{18}O_5{}^{79}BrI$: 519.9382; found [M$^+$] 519.9386.

Combretastatin A-4 (1)

To a cooled solution (–78 °C) of (*Z*)-**8b** (90 mg, 0.17 mmol) in THF (5 mL) was added a 2.5 M hexane solution of *n*-BuLi (0.3 mL, 0.75 mmol) over 2 min. After 30 min at –78 °C the solution was allowed to warm to r.t. then H_2O (5 mL), sat. NH_4Cl (5 mL) and EtOAc (10 mL) were added. The aqueous phase was separated and extracted with EtOAc (5 × 10 mL). The combined organic phases were then dried ($MgSO_4$), concentrated in vacuo and purified by column chromatography (SiO_2, 30% EtOAc–PE × 2) to give combretastatin A-4 (*Z*)-**1** (49 mg, 0.16 mmol, 90%) as a pale yellow solid.[11]

Acknowledgment

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References and Notes

(1) Combretastatin A-4 was isolated from the bark of the African bush willow, *Combretum caffrum*. See: 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.

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(10) Supplementary information providing characterization data for each of the (Z)-stilbenes reported herein, together with details of their preparation and the method of purification employed, is available from the author.

(11) All spectral and physical data were in close agreement with literature values,[1,3] except for the mp, (lit.[1] 116 °C). Following recrystallization of our synthetic sample from either EtOAc or EtOAc–hexane, it gave mp 83–84 °C (recorded in duplicate on two separate apparatuses).