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A practical radiosynthesis of a tritium-labelled fluorocombretastatin

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Zybrestat (combretastatin A-4 disodium diphosphate) is currently in clinical trials as an antivascular anticancer agent. A similar fluorinated agent has shown promise as an antivascular agent, and a radiolabelled version would enable further understanding of its biological activities. Herein is described the synthesis of a tritiated fluorinated analogue of Zybrestat.

Keywords: Zybrestat; combretastatin; tritiation

Introduction

Combretastatin A-4 phosphate **2** (CA4P, Zybrestat) and combretastatin A-1 phosphate **4** (CA1P, OXi4503) (Figure 1) are presently in Phase III and Phase I clinical trials for cancer, respectively.[1] CA4P is also in Phase II clinical trial for age-related macular degeneration[1] (a major cause of blindness for those aged over 50 years). These two agents, along with AVE8062 (Ombrabulin)[2] and BNC105,[3] are the leading cancer vascular damaging drugs presently in clinical trials. The availability of a biologically active radiolabelled combretastatin would greatly enhance the understanding of the pharmacokinetics and pharmacodynamics of these types of molecules. Methods for the syntheses of radiolabelled analogues of CA4P[4] and CA1P[5] have been developed, and the synthesis of ^{14}C-labelled combretastatin A-1 (CA1) and CA1P has been accomplished.[6]

A fluorinated derivative (**5**) of combretastatin A-4 **1** is also a potent tubulin inhibitor,[7,8] which shows antitumour effects *in vivo* (Figure 2). Magnetic resonance imaging studies show that this fluorocombretastatin also damages tumour vasculature (Hadfield JA and McGown AT, unpublished). A radiolabelled version of this fluorocombretastatin will provide a useful tool to understand the pharmacokinetics and pharmacodynamics of this type of agent. Also, a radiolabelled fluorocombretastatin could replace tritiated colchicine in a tubulin colchicine displacement assay[9] and would enable a direct comparison between the binding affinities for tubulin of combretastatin A-4 and the fluorocombretastatin. This paper describes the synthesis of a tritiated fluorocombretastatin.

Results and discussion

The radiolabelling strategy adopted involves the methylation of a free phenolic group on a *cis*-stilbene using tritiated methyl iodide. Methylation was carried out on the A-ring 3-position as the initial formation of a phenoxide on either the 4-position or the 4′-position would be expected to lead to *Z* to *E* isomerisation of the alkene.[5] For related examples, this is thought to be due to delocalisation of the negative charge of the intermediate phenoxide anion, which can be delocalised over the aromatic system and onto the olefin bond of the stilbene. In one resonance form, the anion is localised at the benzylic position. This allows rotation around the single bond in this resonance form leading to a more favoured *trans* configuration as illustrated in Figure 3.

Before attempting the radiosynthesis of the desired fluorinated combretastatin, model reactions were performed using 'cold' methylating agents. The Wittig reaction of 3-fluoro-4-methoxybenzaldehyde **6** with phosphonium bromide **7** (ref.[10]) afforded the two silyl ethers, **8** and **9**, in 41% and 29% yield, respectively, following separation by column chromatography (Figure 4). Deprotection of the silyl ethers **8** and **9** using tetrabutylammonium fluoride (TBAF) gave the *Z* and *E* phenols **10** and **11** in high yields, respectively. The compounds were obtained isomerically pure as indicated by their olefinic coupling constants in their ^{1}H NMR spectra. For example, stilbene **11** is configured *E* as indicated by the signals at δ 6.84 and 6.90 ppm, which have a coupling constant of 16.2 Hz. The corresponding *Z* isomer **10** olefinic protons are observed as a 2 H singlet at higher field (δ 6.45 ppm). For **8** and **9**, the olefinic signals are observed as 2 H singlets at 6.86 (*E*) and 6.44 ppm (*Z*) consistent with previous findings[8,11,12] that the olefinic signals of stilbenes of the *Z* isomer are observed at higher field.

The next stage required an efficient procedure for the methylation of the *Z* phenol **10** with methyl iodide. Following the method of Songca[13] methyl iodide was added to a solution of the phenol **10** and potassium carbonate in dimethylformamide (DMF). After

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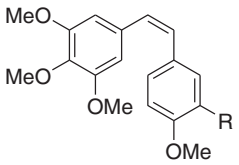
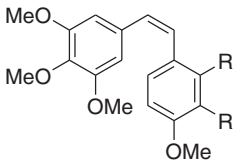
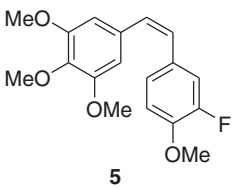


1 Combretastatin A-4 R = OH
2 Combretastatin A-4P R = $OP(=O)(ONa)_2$



3 Combretastatin A-1 R = OH
4 Combretastatin A-1P R = $OP(=O)(ONa)_2$

Figure 1. Structures of Combretastatins.



5

Figure 2. Structure of Fluorocombretastatin **5**.

1 h, the reaction was complete (determined by TLC), and after work up, the desired *Z*-1-(3′,4′,5′-trimethoxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene **5** was obtained in 95% yield (Figure 5).

However, DMF is a difficult solvent to remove owing to its high boiling point, so the methylation was attempted using more volatile solvents. Nyemba[14] reported using acetone under reflux with potassium carbonate and methyl iodide for the methylation of the phenols. When this reaction was performed with a large excess (10 mol equivalents) of potassium carbonate at room temperature, the methylation was complete after 4 h. The longer time for the reaction and the need for a large excess of base were largely attributed to the poor solubility of the potassium carbonate in acetone. This reaction time was shortened by changing the solvent to acetonitrile: the reaction was then complete after 2 h. Even so, the use of a boiling solvent needs to be avoided if possible when handling radioactive materials, so another method was sought.

It has been reported[15] that fluoride impregnated on alumina is an effective reagent for promoting the alkylation of phenols and alcohols. Indeed, when the reaction of methyl iodide and the phenol **10** was performed using acetonitrile as the solvent and potassium fluoride on alumina as the base, the reaction was complete in 30 min. Filtering the product through a short silica column afforded the fluorostilbene **5** in high yield (96%). Key diagnostic signals in the ^{1}H NMR spectrum confirmed the presence of the extra methoxy group (3.72 ppm), and observation of the olefinic coupling constants (*J* 12.4 Hz) confirmed that no isomerisation to the *E* stilbene had occurred.

The reaction was then carried out using tritiated methyl iodide (3.52 μCi) under Ando's reaction conditions.[15] (Figure 6) Cold methyl iodide was added at the end of the experiment to ensure that the reaction went to completion. The fluorostilbene **12** was isolated in 98% yield (by mol). This was single spot by TLC (UV and radio detection) and co-ran with unlabelled compound **5**. Determination of the number of decays per minute indicated 75% tritium incorporation into the molecule.

Experimental

General

All reagents and chromatography grade solvents were obtained from commercial sources and used without further purification unless indicated. Flash column chromatography was performed on silica gel [Fluka, silica gel 60 220–440 mesh (35–70 μm)], and TLC was carried out using silica (0.2 mm, 60 F_{254}) pre-coated, aluminium-backed plates. Mass spectra were recorded on VG70-70 EQ (FAB, CI^+, EI^+) and MS50 (FAB) spectrometers, with only major peaks being reported. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were carried out by the Microanalytical Department at UMIST and Manchester University and are within 0.3% of theoretical values. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AC-300 instrument or at 400 MHz on a Bruker AC-400 MHz instrument. ^{13}C NMR spectra were recorded at either 75.5 MHz on a Bruker AC-300 instrument or at 100 MHz on a Bruker AC-400 instrument. Chemical shifts (δ) are quoted in ppm, relative to tetramethylsilane and are referenced to the $CDCl_3$ unless otherwise stated. Coupling constants (*J*) are reported to 1 decimal place.

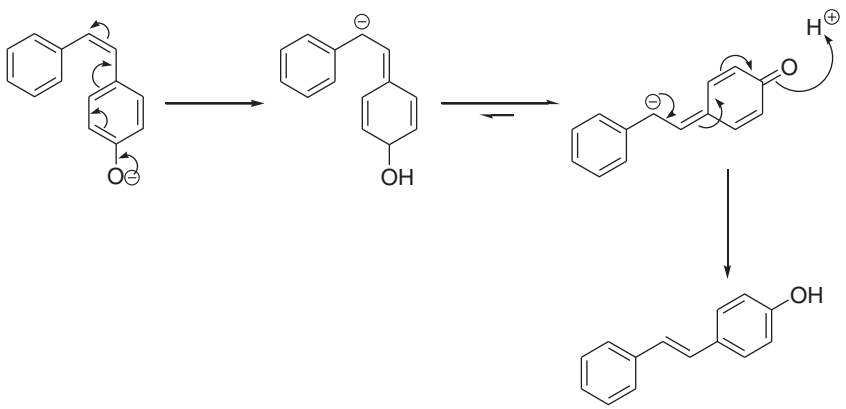


Figure 3. Z to E Isomerisation Mechanism of 4-Hydroxystilbenes.

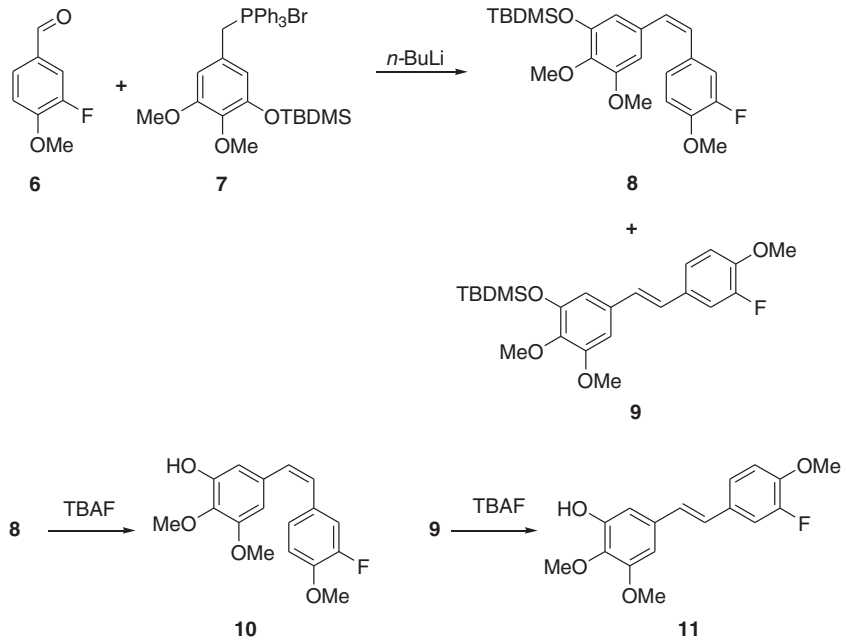


Figure 4. Synthesis of Phenols 10 and 11.

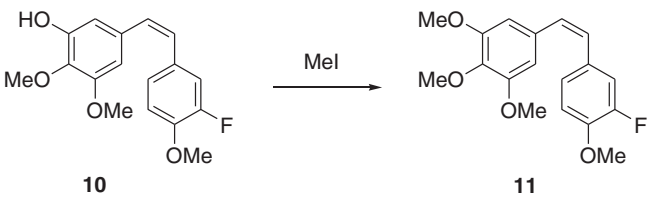


Figure 5. Methylation of 10.

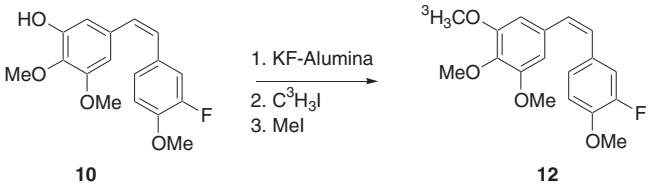


Figure 6. Synthesis of tritiated fluorocombretastatin **12**.

*Z-1-(3′,4′-Dimethoxy-5′-t-butyldimethylsilyloxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene **8***

To a slurry of the phosphonium bromide **7** (3.94 g, 6.331 mmol) in THF (30 cm^3) was added ^{n}BuLi (5 cm^3 of 1.6 M solution, 8.0 mmol) at −15 °C. The bright red anion was stirred for 20 min before 3-fluoro-4-methoxybenzaldehyde **6** (1.0 g, 6.487 mmol) was added. The mixture was stirred for 1 h. Water was added to the mixture, and the resulting solution was extracted with diethyl ether (3 × 60 cm^3). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to furnish a brown oil. This was purified by flash column chromatography (SiO_2, ethyl acetate : hexane, 1:20 v/v) to furnish **8** as a yellow oil (1.12 g, 41%). Found: C, 65.8; H, 7.5. M^+, 418.1979. $C_{23}H_{31}FO_4Si$ requires C, 66.0; H, 7.5%; 418.1975. R_f 0.82 (SiO_2, petrol : EtOAc 4:1 v/v); δ_H ($CDCl_3$; 300 MHz) 0.10 [6 H, s, Si-$(CH_3)_2$], 0.96 [9 H, s, C$(CH_3)_3$], 3.72 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 6.40 (1 H, d, *J* 2.0 Hz, H-6′), 6.44 (2 H, s, CH=CH), 6.47 (1 H, d, *J* 2.0 Hz, H-2′), 6.84 (1 H, t, *J* 8.7 Hz, H-5″), 6.99 (1 H, dd, *J* 1.8 and 8.7 Hz, H-6″), 7.04 (1 H, dd, *J* 1.8 and 12.3 Hz, H-2″).

*E-1-(3′,4′-Dimethoxy-5′-t-butyldimethylsilyloxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene **9***

Further elution afforded **9** as an off-white solid (0.57 g, 29%). Found: C, 65.9; H, 7.4. M^+, 418.1974. $C_{23}H_{31}FO_4Si$ requires C, 66.0; H, 7.4%; 418.1975. m.p. 117–119 °C. R_f 0.75 (SiO_2, hexane : ethyl acetate 4:1 v/v); δ_H ($CDCl_3$; 300 MHz) 0.226 [6 H, s, Si-$(CH_3)_2$], 1.04 [9 H, s, C$(CH_3)_3$], 3.92 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 6.64 (1 H, d, *J* 2.1 Hz, H-6′), 6.70 (1 H, d, *J* 2.1 Hz, H-2′), 6.86 (2 H, s, CH=CH), 6.94 (1 H, t, *J* 8.4 Hz, H-2″), 7.18 (1 H, dd, *J* 1.7 and 8.4 Hz, H-6″), 7.30 (1 H, dd, *J* 1.7 and 12.9 Hz, H-2″).

*Z-1-(3′,4′-Dimethoxy-5′-hydroxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene **10***

To a stirred solution of the *Z* silyl ether **8** (0.949 g, 2.27 mmol) in dry THF (25 cm^3) was added (TBAF) (6 cm^3 of 1.0 M solution in THF, 6 mmol). The resulting yellow solution was stirred for 20 min and then treated with water (50 cm^3). The mixture was extracted with chloroform (3 × 25 cm^3) and the combined organic extracts washed with water (2 × 25 cm^3), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting colourless oil was purified by flash column chromatography (SiO_2, ethyl acetate : hexane, 1:4 v/v) to yield the stilbene **10** as a yellow oil (609 mg, 100%). Found: M^+, 304.1113. $C_{17}H_{17}FO_4$ requires 304.1111. R_f 0.57 (SiO_2, petrol : EtOAc 1:1 v/v); δ_H ($CDCl_3$; 400 MHz) 3.70 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 5.72 (1 H, s, OH), 6.41 (1 H, d, *J* 1.4 Hz, H-6′), 6.45 (2 H, s, CH=CH), 6.54 (1 H, s, *J* 1.4 Hz, H-2′), 6.85 (1 H, d, *J* 8.4 Hz, H-5″), 7.01 (1 H, dd, *J* 2.0 and 8.4 Hz, H-6″), 7.07 (1 H, dd, *J* 2.0 and 12.4 Hz, H-2″).

*E-1-(3′,4′-Dimethoxy-5′-hydroxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene **11***

In a similar manner, the *E* silyl ether **9** (0.662 g, 1.58 mmol) was deprotected using TBAF (3 cm^3 of 1.0 M solution in THF, 3 mmol) to yield **11** as a white solid (398 mg, 83%) after flash column chromatography (SiO_2, ethyl acetate : hexane, 1:4 v/v). Found: C, 67.0; H, 5.7. M^+, 304.1116. $C_{17}H_{17}FO_4$ requires C, 67.1; H, 5.6%; 304.1111. m.p. 132–134 °C. R_f 0.56 (SiO_2, petrol : EtOAc 1:1 v/v); δ_H ($CDCl_3$; 300 MHz) 3.92 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 3.94

(3 H, s, OCH_3), 5.79 (1 H, s, OH), 6.61 (1 H, d, *J* 2.3 Hz, H-6′), 6.78 (1 H, d, *J* 2.3 Hz, H-6′), 6.84 (1 H, d, *J* 16.2 Hz, C=CH), 6.90 (1 H, d, *J* 16.2 Hz, C=CH), 6.95 (1 H, t, J 8.7 Hz, H-5″), 7.18 (1 H, dd, *J* 2.7 and 8.7 Hz, H-6″), 7.27 (1 H, dd, *J* 2.7 and 12.3 Hz, H-2″).

Z-1-(3′,4′,5′-Trimethoxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl) ethene ***5***

Following a similar method with that of Ando and co-workers,[15] the phenol **10** (10 mg, 0.032 mmol) was stirred with KF-alumina (25 mg) in acetonitrile (1 mL) for 20 min before iodomethane (3 μL, 0.042 mmol) was added. This was stirred for 30 min before being run down a short silica column, eluting with ethyl acetate. Evaporation of the solvent afforded the fluorocombretastatin **5** as a white solid (10 mg, 96%). m.p. 76–78 °C (ref.[8] 75–78 °C); R_f 0.62 (SiO_2, hexane : ethyl acetate 1:1 v/v); δ_H ($CDCl_3$; 300 MHz) 3.72 (6 H, s, 2 × OCH_3), 3.72 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 6.42 (1 H, d, *J* 12.4 Hz, C=CH), 6.49 (1 H, d, *J* 12.4 Hz, C=CH), 6.51 (2 H, s, H-2′ and H-6′), 6.85 (1 H, t, *J* 8.3 Hz, H-5″), 7.01 (1 H, dd, *J* 2.4 and 8.3 Hz, H-6″), 7.07 (1 H, dd, *J* 2.4 and 12.6 Hz, H-2″).

[^{3}H]Z-1-(3′[^{3}H],4′,5′-Trimethoxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene ***12***

The phenol **10** (7 mg, 0.023 mmol) was stirred with KF-alumina (18 mg) in acetonitrile (1 mL) for 20 min before [^{3}H]iodomethane (800 μL, 3.52 μCi, 5.5×10^{-8} M in toluene, specific activity: 80 Ci/mmol) was added. This was stirred for 4 h before iodomethane (3 μL, 0.042 mmol) was added. The mixture was allowed to stir for a further 30 min before being evaporated to dryness and eluting down a short silica column with ethyl acetate to furnish **12** as a white solid (7 mg, 2.64 μCi, 75% incorporation). R_f 0.61 (SiO_2, hexane : ethyl acetate 1:1 v/v).

Conclusions

In conclusion, we have developed three methods for the potential incorporation of a radiolabel into the fluorocombretastatin **5**. One of these methods was used to successfully incorporate a tritium label into the molecule. Also, with a quick (0.5 h) reaction time, it should be feasible to incorporate an ^{11}C label for use in positron emission tomography studies using this methodology.

Acknowledgements

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Conflict of Interest

The authors did not report any conflict of interest.

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