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Research Article

Synthesis of (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene

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Abstract: In this paper, we describe the radiosynthesis of the compound (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene, a potential, universal tumour positron emission tomography imaging agent. The production of (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene was carried out via ^{11}C-methylation of (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene by using [^{11}C]methyl trifluoromethanesulfonate ([^{11}C]methyl triflate). (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene was obtained with a radiochemical purity greater than 95% in a 20 ± 2% decay-corrected radiochemical yield, based upon [^{11}C]carbon dioxide. Synthesis, purification and formulation were completed on an average of 30 min following the end of bombardment (EOB). The specific radioactivity obtained was 1.9 ± 0.6 GBq/μmol at EOB. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: 2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene; CYP1B1; PET

Introduction

Over the last decade positron emission tomography (PET), using the glucose metabolism-imaging agent 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG), started to play a major role in the management of patients, especially in oncology.[1] Although FDG-PET is invaluable in the imaging of a number of tumour types, it is not suitable for detection of all tumours due to the high uptake of FDG by normal tissues,[2,3] inability to differentiate some low/medium grade tumours from benign lesions,[4] limited use in the diagnosis of some recurrent tumours,[5] as well as accumulation of FDG in inflammation making it indistinguishable from tumour tissue. Hence, other PET tracers with greater tumour specificity need to be developed. Malignant tumour cells can abnormally express or overexpress proteins including enzymes which are consequently potential targets for imaging with radiolabelled substrate analogues. One approach is to label inhibitors of the enzyme to which it becomes associated with and disassociate only very slowly.[6] To be useful as a PET tracer, due to the short half-lives of the radioisotopes (e.g. ^{11}C $t_{1/2} = 20.4$ min), the molecule must be capable of being rapidly radiolabelled. A recently discovered member of the cytochrome P450 (CYP) family is the protein P450 1B1, which is a major oestrogen (E2) 4-hydroxylase and is involved in the metabolic activation of several polycyclic aromatic hydrocarbon carcinogens including benzo(a)pyrene, dibenzo(a,l)pyrene, 7,12-dimethylbenz(a)anthracene and 5-methyl chrysene.[7] Although CYP1B1 mRNA has been found in a number of normal human tissues, the presence of the protein appears to be specific to tumour tissue.[8] Using immunohistochemistry, Murray *et al.*[9] found that CYP1B1 was absent in all 130 samples of normal human tissue from 16 different anatomical regions. In contrast, CYP1B1 protein was present in 122 of 127 samples of malignancies from each of the 16 anatomical regions. Further, immunoblotting and immunochemistry failed to reveal the presence of native CYP1B1 protein in human liver[8] and only very low levels in cells prepared from non-neoplastic breast tissue,[10,11] but immunoblotting[12] and immunochemistry[9] consistently demonstrated the presence of CYP1B1 in human breast tumours. In 2002, a number of inhibitors of CYP1B1 have been developed,[13] the most selective of which is (*E*)-2,3′,4,5′-tetramethoxystilbene (TMS). Kim *et al.* discovered that TMS inhibited P450 1B1 with a 50-fold selectivity over P450 1A1 and 500-fold selectivity over P450 1A2-, the other two members of the CYP enzyme family.[13] This inhibitor is metabolized by P450 1B1 at a very slow rate, so that the molecule can be labelled in any position.[14] Here, we report the synthesis of the ^{11}C

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isotope-radiolabelled CYP1B1 inhibitor (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene.

Results and discussion

To achieve our aim, the ^{11}C radiolabelling of the non-radioactive precursor (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene, **3**, the synthetic strategy published by Kim *et al.*[13] was examined. Kim *et al.* reported a synthetic pathway to produce a series of *trans* stilbene derivatives and to evaluate their inhibitory activities on the human cytochrome P450s.[13] It was discovered that the substituent at position 2 of the stilbene skeleton (Figure 1) plays an imperative role in discriminating between CYP1B1 and other P450 s CYP inhibitors.[13] Despite following the described synthetic method using the same conditions as Kim *et al.*, the targeted product **3** could not be obtained. Hence, a slightly modified synthetic route was adopted by ChiroBlock GmbH for us. The synthetic scheme for the synthesis of (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene, **3**, is presented in Figure 2. 3,5-Dimethoxybenzyltriphenylphosphonium bromide **1** was synthesized with a yield of 93% according to a procedure for the preparation of phosphonium salts.[15] For this, 3,5 dimethoxybenzyl-bromide was dissolved in xylene and charged with triphenylphosphane. The next step involved the protection of the hydroxy group of 2-hydroxy-4-methoxy-benzaldehyde. Experiments to carry out the subsequent olefination reaction, and to construct the stilbene skeleton, by reacting unprotected 2-hydroxy-4-methoxy-benzaldehyde[13] with **1** were unsuccessful. Therefore, the facile removable allyl-protecting group was chosen. 2-Hydroxy-4-methoxy-benzaldehyde was charged with allylbromide to obtain 2-allyloxy-4-methoxy-benzaldehyde **2** with a yield of 37%. The subsequent reaction between **1** and **2** resulted in the unprotected isomer (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene **3**. For this, **1** was reacted with sodium hydride and **2**. The product isolated was reacted with sodium borohydride and tetrakis(triphenylphosphane) palladium(0). The purified product was dissolved in heptane and reacted with iodine. Column chromatography provided pure **(***E***)**-2-(hydroxy)-3′,4,5′-trimethoxystilbene **3** with a yield of 14%. The radiosynthesis of (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene, **4**, is presented in Figure 3. Compound **4** has been successfully prepared in a single-step reaction by ^{11}C-methylation of **3** using [^{11}C]methyl triflate. For this, a Pyrex 10 ml test tube was filled under an argon atmosphere with a solution of **3** in acetonitrile and potassium carbonate and placed into a dose calibrator (Capintec CRC-15PET). Once the amount of [^{11}C]methyl triflate bubbling through the yellow suspension reached a maximum, the reaction mixture was worked up. Isolation and purification of **4** were accomplished by means of a small disposable C-18 cartridge. After the yellow mixture was removed from the test tube and introduced to the C-18-based resin, the cartridge was rinsed with sterile water. The product **4** was eluted with 1.5 ml of ethanol from the cartridge

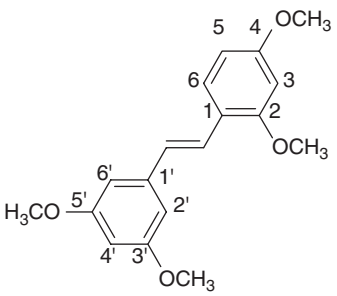

Figure 1 Chemical structure of (*E*)-2,3′,4,5′-tetramethoxystilbene.

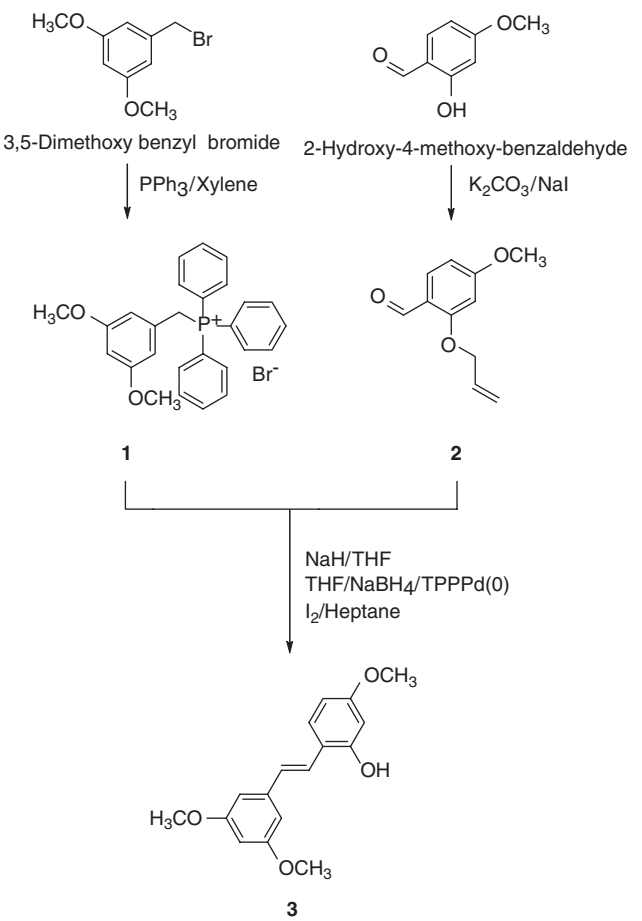

Figure 2 Synthesis of (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene **3**.

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through a sterile 0.22 Micron Filter (Pall) into a sterile nitrogen-filled vial containing 9 ml of sterile sodium chloride, 0.9% w/v, solution. Analytical high-performance liquid chromatography (HPLC) showed the product to be >95% radiochemically pure in a $20 \pm 2\%$ decay-corrected radiochemical yield (based upon [^{11}C]carbon dioxide) and to co-elute with a sample of non-radioactive TMS at the same retention time of 20 min (Figure 4). The total synthesis time from end of bombardment (EOB) was 30 min and the specific activity of **4** was 1.9 ± 0.6 GBq/μmol at EOB. Recently, a research group working independently on a similar synthetic approach reported the synthesis of ^{11}C- and ^{18}F-labelled *trans* stilbenes. Gao *et al.*[16] described, among other compounds, the synthesis of (*E*)-3,3′,4,5′-tetramethoxy[3-^{11}C]stilbene and (*E*)-3′,4,5′-trimethoxy[4-^{11}C]stilbene as potential probes for aryl hydrocarbon receptor in cancers. However, none of their labelled stilbene derivatives had a methoxy group at position 2 of the stilbene skeleton and would, therefore, have a lower affinity for CYP1B1 compared with the ^{11}C-labelled compound that we have produced.

Experimental

General

Pure (>98%) TMS was purchased from Cayman. Pure (>95%) (*E*)-2-(hydroxy)-3′,4,5′-trimethoxystilbene was custom synthesized by ChiroBlock GmbH. All other reagents were purchased from Sigma-Aldrich and were used without further purification unless otherwise noted. All used solvents were purified and degassed according to standard procedures. FT-IR spectra were recorded using a Nicolet 205 FT-IR spectrometer. Mass spectra were recorded on a Shimadzu QP5050 mass spectrometer ^{1}H and ^{13}C NMR data were obtained on a Varian Unity Inova 400 MHz and Bruker 400 MHz spectrometer at 300 K. Chemical shifts are given in ppm. Melting point analyses were carried out using a Gallenkamp melting point apparatus. Analytical HPLC was performed using a Gynkotek HPLC system (P580 pump), a Gynkotek column oven (STHS8S) and a Gynkotek UV detector (UVD340S) coupled in series with a BIOSCAN NaI detector (B-FC-3200). The HPLC system was operated using a Phenomenex Luna C-18 column (250 × 3.0 mm, particle size: 5 μm). The eluent used was a mixture of HPLC grade acetonitrile and 0.1 M ammonium formiate solution (50:50). The eluent

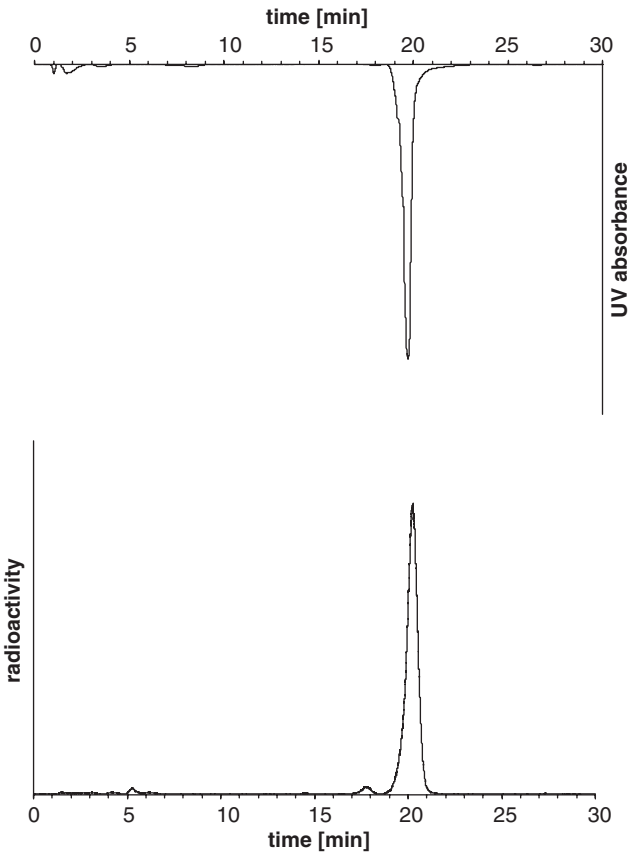
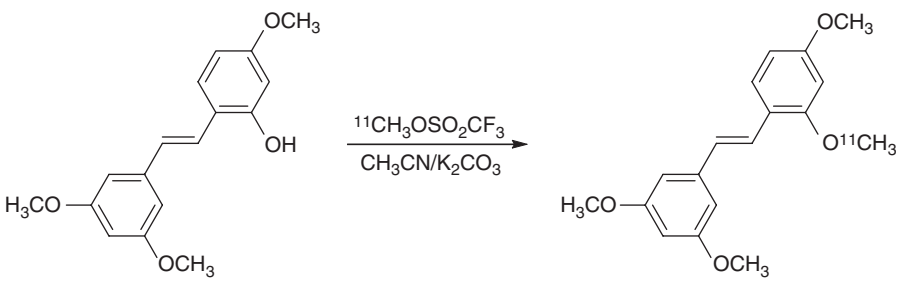


Figure 4 Top: UV-chromatogram of non-radioactive (*E*)-2,3′,4,5′-tetramethoxystilbene **4**. Bottom: Radioactivity chromatogram of (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C] stilbene (97%, 20.1 min). Both peaks at 5.1 and 17.6 min are unidentified.



3 **4**

Figure 3 Radiosynthesis of (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C] stilbene **4**.

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was filtered and degassed with Grade A helium (BOC) before use. The flow rate was set at 1.2 ml/min. The column oven was set at 40°C. [^{11}C]Carbon dioxide, [^{11}C]methyl iodide and [^{11}C]methyl trifluoromethanesulfonate radiosyntheses have been carried out according to procedures described previously.[17]

3,5-Dimethoxybenzyltriphenylphosphonium Bromide (1)

To a stirred solution of 3,5-dimethoxybenzyl bromide (5.0 g, 0.02 mol) in 40 ml of xylene, a solution of triphenylphosphane (5.96 g, 0.02 mol) in 10 ml of xylene was added dropwise. The orange suspension was refluxed overnight. The flask was cooled down to r.t. and then placed in the freezer overnight. The precipitation was filtered, washed with cold xylene and dried. 3,5 Dimethoxybenzyltriphenylphosphonium bromide, **1** was obtained as beige crystals (9.95 g, 93%). M.p. 274–275°C (lit.[18]: m.p. 275°C). ^{1}H NMR: (400 MHz, $CDCl_3$): 7.74–7.57 (m, $3 \times C_6H_5$, 15 H), 6.29 (d, H-2, H-6, $J = 2.4$ Hz, 2 H), 6.25 (t, H-4, $J = 2.4$ Hz, 1H), 5.25 (d, $-CH_2$, $J = 14$ Hz, 2H), 3.49 (s, $2 \times -OCH_3$, 6 H).

2-Allyloxy-4-methoxy-benzaldehyde (2)

To A mixture of 2-hydroxy-4-methoxy-benzaldehyde (3.00 g, 0.02 mol) and allylbromide (2.39 g, 0.02 mol), potassium carbonate (K_2CO_3) (4.09 g, 0.03 mol) and a catalytic amount of sodium iodide (3.0×10^{-3} g, 2.0×10^{-7} mol) was added. The reaction mixture was stirred at r.t. overnight. After 12 h, the yellow mixture was heated up to 40°C and stirred for 18 h. Twenty milliliters of acetone was added to the brown solution and stirred for an additional 20 h at 40°C. After concentration of the mixture, the residue was dissolved in dichloromethane. Purification by column chromatography on silica gel (ethylacetate/petrolether, 1:7) provided 2-allyloxy-4-methoxy-benzaldehyde **2** as a white solid (1.4 g, 37%). M.p. 38°C (lit.[19]: m.p. 37.9–38.1°C). ^{1}H NMR: (400 MHz, $CDCl_3$): 10.29 (s, CHO, 1H), 7.75 (d, H-6, $J = 8.4$ Hz, 1H), 6.48 (dd, H-5, $J = 8.4$, 2.4 Hz, 1H), 6.37 (d, H-3, $J = 2.4$ Hz, 1H), 6.04–5.97 (m, $OCH_2CH{=}CH_2$, 1H), 5.41–5.26 (m, $OCH_2CH{=}CH_2$, 2H), 4.57–4.55 (m, $OCH_2CH{=}CH_2$, 2H), 3.80 (s, $-OCH_3$, 3H), (cf. Reference 19).

(E)-2-(hydroxy)-3′,4,5′-trimethoxystilbene (3)

Compound **1** (3.60 g, 7.0×10^{-3} mol) was added under nitrogen atmosphere to a cooled suspension of sodium hydride (0.25 g, 0.01 mol) in 40 ml of anhydrous tetrahydrofuran (THF). The red suspension was stirred at 5°C for 30 min. A solution of **2** (1.40 g, 0.01 mol) in 10 ml of anhydrous THF was added dropwise. The reaction mixture was stirred at r.t. overnight. After concentration of the mixture, the residue was dissolved in 50 ml of diethylether and washed with water. The water phase was collected and extracted with diethylether. The organic phases were combined, dried over sodium sulfate and concentrated. The obtained yellow oil was dissolved in 50 ml of THF. Then sodium borohydride (0.09 g, 3.0×10^{-3} mol) and tetrakis(triphenyl phosphane)palladium(0) (1×10^{-3} g, 8.7×10^{-7} mol) were added. The yellow solution was stirred at room temperature overnight. After evaporating the solvent, the residue was purified via column chromatography on silica gel (ethyl acetate/petrol ether, 1:2). The product isolated was dissolved in 50 ml of heptane. After adding a catalytic amount of iodine (0.01 g, 3.9×10^{-6} mol), the purple solution was refluxed for 2 h. Concentration of the solution and subsequent purification via column chromatography on silica gel (ethyl acetate/petrol ether, 1:2) provided pure (*E*)-2-(hydroxy)- 3′,4,5′-trimethoxystilbene **3** as an orange viscous oil (0.27 g, 14%). IR (NaCl, film) cm^{-1}: 3390 (–OH), 3050–3000 (CH arom), 2840 (CH–aliph), 1460, 1590 (C=C), 1290, 1030 (C–O). ^{1}H NMR: (400 MHz, ($(CD_3)_2S{=}O$)): 9.77 (s, OH, 1H), 7.41 (d, $J = 9.6$ Hz, 1H), 7.24 (d, =CH–Ph, $J = 16.4$ Hz, 1H), 6.95 (d, =CH–Ph, $J = 16.4$ Hz, 1H), 6.61 (d, $J = 2.1$ Hz, 2H), 6.38–6.31 (m, 3 H), 3.71 (s, $2 \times$ $-OCH_3$, 6H), 3.66 (s, $-OCH_3$, 3H). ^{13}C NMR: (100 Hz, ($(CD_3)_2S{=}O$)): 161.3, 160.6, 156.9, 140.8, 128.3, 126.3, 124.8, 117.4, 106.2, 104.5, 101.9, 99.9, 55.8, 55.7. EIMS *m/z*: 286 (M+, 100%), 137, 255.

(E)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene radiosynthesis (4)

The synthetic strategy for **4** is based on the methodology previously described for the production of [*N*-methyl-^{11}C]methylene blue.[17] (*E*)-2-(hydroxyoxy)-4, 3′,5′-trimethoxystilbene, **3**, (1.0×10^{-3} g, 3.5×10^{-6} mol) was dissolved in 200 µl of anhydrous acetonitrile in a dry Pyrex test tube under argon atmosphere and treated with potassium carbonate (0.01 g, 7.24×10^{-5} mol). The test tube was sealed with a rubber stopper and heated up to 37°C for 2 h. The methylating agent [^{11}C]methyl triflate was bubbled through the test tube via a spinal needle (18 GA, 90 mm). After the amount of [^{11}C]methyl triflate reached a maximum, the reaction mixture was transferred onto a C-18 Sep-Pak Plus cartridge (Waters) which was consequently washed with 15 ml of sterile water. Then the cartridge was rinsed with 1.5 ml of ethanol to provide **4**. Identification of **4** was carried

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out using analytical HPLC. The synthesis of the radiolabelled product **4** was confirmed via co-injection with a commercial sample of TMS. The retention time of TMS in the UV-chromatogram (300 nm) was identical to the retention time of **4** in the radioactivity chromatogram (Figure 4). In a typical radiochemical experiment, 11 GBq of [^{11}C]carbon dioxide could be converted into 0.74 GBq of **4** within an overall synthesis, purification and formulation time of 30 min (from EOB). The decay-corrected radiochemical yield, based on [^{11}C]carbon dioxide, was 20 $\pm$ 5% and the radiochemical purity of **4**, measured by analytical HPLC, exceeded 95%. The specific radioactivity obtained was 1.9 $\pm$ 0.6 GBq/μmol at EOB. Determination of the specific radioactivity of **4** was carried out by means of analytical HPLC according to Mock *et al.*[20]

Conclusions

In conclusion, we have synthesized (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene by means of a ^{11}C-methylation reaction with a radiochemical purity greater than 95% in a 20 $\pm$ 5% radiochemical yield , based on [^{11}C]carbon dioxide, as well as a specific average activity of 1.9 $\pm$ 0.6 GBq/μmol at EOB. To our knowledge, (*E*)-2,3′,4,5′-tetramethoxy[2-^{11}C]stilbene is a new compound which may be a potential, universal tumour *in vivo* PET tracer.

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