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Research Article

No-carrier-added, ^{18}F-labelling of a cholesterol derivative, used in detection of adrenal malignancies

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Summary

[^{18}F]Cholesteryl 4-(fluoromethyl)benzoate **6b** was prepared from 3-cholesteryl [(4-nitrobenzenesulfonyl)oxymethyl]benzoate in one step using K^+-Kryptofix 2.2.2, as counter ion and acetonitrile as the solvent at 40–60°C. The product was isolated by HPLC in 75% (decay corrected) radiochemical yield with a radiochemical purity of more than 90% in 30 min (specific radioactivity 2000–2500 Ci/mmol). Biodistribution studies in mice showed that a high target/non-target ratio of radioactivity was obtained in adrenal and ovaries. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: fluorine-18; PET; cholesteryl esters; adrenal malignancies; [^{18}F]cholesteryl 4-(fluoromethyl)benzoate ([^{18}F]CFMB)

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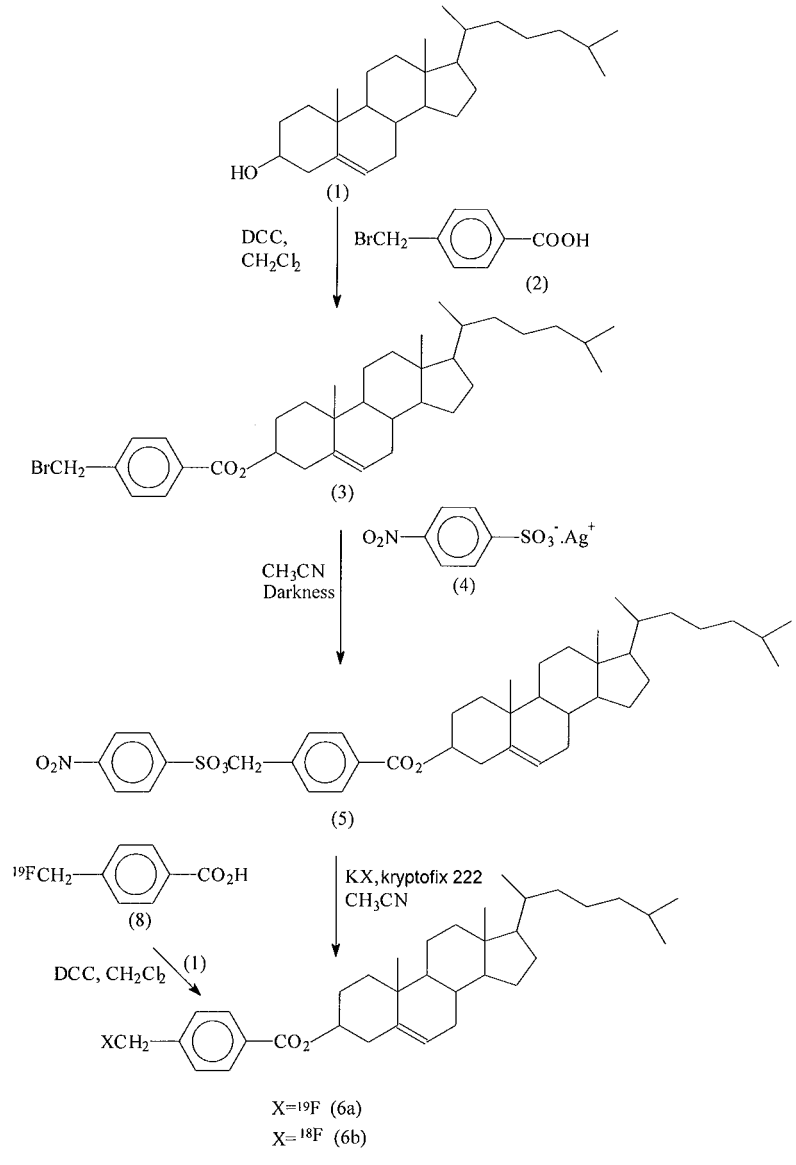
Introduction

Cholesterol derivatives accumulate in steroid secreting tissues because these organs use cholesterol as a precursor in steroid synthesis. Gamma emitting isotopes of iodine (^{131}I; $t_{1/2} = 8$ day or ^{123}I; $t_{1/2} = 13.2$ h) have been used to label cholesterol and its derivatives for administration to human subjects. The labelled compounds were selectively absorbed in adrenal and ovaries.[1,2] Thus, cholesteryl esters can be used as adrenal imaging agents to diagnose various adrenal diseases.[3] Previously, a fluorine-18-labelled cholesteryl ester, [^{18}F]cholesteryl 4-fluorobenzoate ([^{18}F]-CFB) had been prepared by a fluoro for nitro exchange reaction and tested as a prospective radioligand for positron emission tomography (PET).[4,5] In this paper, a new radiolabelled cholesterol ester, [^{18}F]cholesteryl 4-(fluoromethyl)benzoate ([^{18}F]CFMB), has been developed. In view of the low efficacy of fluoro for nitro exchange used in the previous labelling procedure, it was our intention to produce a new cholesteryl aromatic ester that could be labelled in higher yields and under mild conditions.

Results and discussion

Highly sterically hindered cholesteryl esters, when suitably radiolabelled have been shown to be good diagnostic agents, based on their slow rate of hydrolysis and high accumulation rate in steroid secreting tissues.[2,6]

Aliphatic nucleophilic substitution is one of the best and most efficient way to synthesize ^{18}F-labelled ligands. Many research groups have shown that sulfonyl esters are the best candidates for fluoro exchange.[7] The radiolabelled target molecule **6b**, was prepared according to Scheme 1. In the first step, cholesteryl 4-bromomethyl-benzoate was prepared in a rapid procedure with high purity by condensation of related acid **2** and cholesterol **1** using DCC in anhydrous dichloromethane. The labile 4-nitrobenzenesulfonate group had been shown to be the best leaving group for nucleophilic fluoride anion in the presence of reactive aminopolyether, Kryptofix 222, in acetonitrile as solvent. In the next step, in order to obtain a better leaving group than bromo, freshly prepared silver 4-nitrobenzene sulfonate salt **4**, was treated with **3** in acetonitrile at room temperature and darkness according to previous methods.[8]



Scheme 1. Preparation of 6a and 6b

The product, compound **5**, was repeatedly recrystallized to ensure the removal of silver ion, since trace silver impurities are susceptible to light and temperature. The pure compound can be easily stored for several months. The final solution was injected into mice intravenously. Enhanced radioligand uptake was observed 30 min after ethynyl

esteradiol injection as previously reported in the literature.[9–11] After 1 h, the subjects were dissected and the radioactivity of different organs was measured using a radiometer. Previous studies had shown that lipoprotein uptake in adrenal regions was enhanced after ethinyl esteradiol pretreatment.[12] Due to cholesterol derivative incorporation into lipoproteins, their absorption is increased using ethynyl esteradiol. In this study, ethynyl esteradiol pretreatment effectively increased the adrenal uptake 5 fold and spleen uptake by greater than 16 fold. Uptake of radioactivity by bone was low in the control and in ethinyl esteradiol pretreated groups due to low defluorination of ^{18}F-CFMB.

Experimental

Cholesterol, Kryptofix 222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo [8,8,8] hexacosane) and other chemicals were purchased from the Aldrich Chemical Co., UK. ^{1}H NMR spectra were obtained on a Bruker FT-80 (80 MHz) or a Varian (400 MHz) instrument with tetramethylsilane as the internal standard. Infrared spectra were taken on a Perkin-Elmer 781 spectrometer (KBr disks). Mass spectra were recorded using a Finnigan Mat TSQ-70 spectrometer. Thin-layer chromatography (TLC) of non-radioactive products was performed on silica gel polymer backed (F 1500/LS 254, $20 \times 20\,cm^2$, TLC Ready Foils Schleicher & Schuell™) or glass plates ($25 \times 35\,cm^2$, E-Merck). Acetonitrile, used for the labelling step was of 'Sure-Seal'™ grade supplied by Aldrich. Analytical HPLC was performed to determine specific radioactivity, with a Shimadzu LC-10AT instrument, equipped with two detector systems, that included a flow scintillation analyzer (with Packard-150TR) and UV-visible (Shimadzu) detector using on Si Kromasil 100, 5 μm $250 \times 4.6\,mm^2$ (M & W), Inchrom™ column. The specific radioactivity of **6b** was calculated using a standard curve prepared for **6a**. Radiochromatography was performed using a rotary motor coupled to a Canberra™ germanium detector (model GC1020-7500SL) using polymer-backed silica gel paper. Purification of **6b** was performed using a Silica Kromasil preparative column purchased from Waters™. Melting points were determined on a Reichert-Jung™ microscope and are not corrected. Elemental microanalyses were within $\pm$ 0.4% of theoretical values for C, H, F, and N.

Preparation of [^{18}F]-potassium fluoride from [^{18}O]-water

[^{18}F]fluoride was prepared by bombardment of an enriched ^{18}O-water sample (1.7 ml, >95 at%, Cortec^{TM}, France) held in an all-silver target with 18 MeV protons from a 30 MeV cyclotron at the Nuclear Research Center for Agriculture and Medicine (NRCAM) Karaj-Iran. After the recovery of ^{18}O-water over an anion exchange membrane (Bio-Rad^{TM} AG 1-X8 Anion exchange, carbonate form 47 mm), fluoride-18 anion was eluted by a 1% potassium carbonate solution (1%, 100 µl). The eluted solution was directly used in the labelling reaction.

Preparation of 4-fluoromethylbenzoic acid 8

Kryptofix 222 (140 mg, 0.38 mmol), methyl 4-bromomethyl benzoate[8] (150 mg, 6.6 mmol) and potassium fluoride (60 mg, 1.05 mmol) were refluxed in anhydrous acetonitrile (10 ml) at 50°C for 15 h. The reaction was monitored by TLC, using hexane as the mobile phase. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuum and purified by TLC over silica gel coated glass plates using hexane as the mobile phase. The fraction at $R_f = 0.3$ was separated and extracted by chloroform. Then the extract was concentrated in vacuum. The physicochemical data were as reported previously.[13] The product can be used in the next step directly. 4-Fluoromethyl benzoic acid **8** was prepared by equimolar hydrolysis of related ester with 1 N sodium hydroxide solution and subsequent acidification. The physicochemical data were as reported previously.[13]

Preparation of cholesteryl 4-bromomethylbenzoate 3

A mixture of 4-bromomethylbenzoic acid **2** (215 mg, 1 mmol), cholesterol **1** (386 mg, 1 mmol) and dicyclohexyl carbodiimide (DCC) (206 mg, 1 mmol) was stirred vigorously in anhydrous dichloromethane (15 ml) for 24 h at room temperature. The mixture was filtered and the filtrate evaporated to dryness. The residue was crystallized from ethyl acetate–hexane mixture (7 : 3, v/v) to give a white light powder (70%). m.p.: 177–180°C. ^{1}H NMR ($CDCl_3$) δ (ppm) 7.48–8.17 (ABq, 4 H, aromatic), 5.30–5.34 (m, 1 H, C_6-H), 4.52 (s, 2 H, CH_2), 3.33–3.37 (m, 1 H, C_3-H), 0.67–2.35 (m, 43 H, aliphatic). IR (KBr) ν_{max} 2967, 1640, 1243, 640, 729.

Preparation of cholesteryl 4-fluoromethylbenzoate 6a

Compound **6a** was prepared according to the procedure discussed above for **3**, using equimolar portions of acid **8**, cholesterol **1** and DCC in anhydrous dichloromethane and led to a yellow oil (60%). ^{1}H NMR ($CDCl_3$) δ (ppm) 7.49–8.27 (ABq, 4 H, aromatic), 5.50 (s, 1 H, FCH_2), 5.33–5.37 (m, 1 H, C_6-H), 5.29 (s, 1 H, FCH_2), 3.48–3.53 (m, 1 H, C_3-H), 0.61–2.31 (m, 43 H, aliphatic). IR (KBr) ν_{max} 3050, 1642, 1245.

Preparation of cholesteryl 4-[(4-nitrobenzene sulfonyl)-oxymethyl] benzoate 5

Compound **5** was synthesized using silver 4-nitrobenzene sulfonate salts **4** and **3** in acetonitrile at room temperature. Freshly prepared silver 4-nitrobenzenesulfonate (0.85 g, 2.7 mmol) was dissolved in Sure-Seal^{TM} grade acetonitrile (10 ml) and the mixture was added to **3** (560 mg, 0.96 mmol) in a capped vial and stirred vigorously at room temperature for 15 h. Then the mixture was filtered and the solvent was evaporated in vacuum. the residue was redissolved in hot ethyl acetate and passed through a small silica column. The column extract was concentrated in vacuum and crystallized from dichloromethane–hexane mixture (2 : 3, v/v). White fine crystals were obtained. m.p.: 108–111°C (43%). ^{1}H NMR ($CDCl_3$) δ (ppm) 8.12–8.43 (ABq, 4 H, aromatic), 7.45–7.53 (ABq, 4 H, aromatic), 5.27–5.30 (m, 1 H, C_6-H), 5.21 (s, 2 H, CH_2), 3.45–3.49 (m, 1 H, C_3-H), 0.65–2.23 (m, 43 H, aliphatic). IR (KBr) ν_{max} 3114, 1699, 1354, 1541, 1150, 1400.

Fluorination of cholesteryl 4-[(4-nitrobenzenesulfonyl)oxymethyl]benzoate 5 with potassium fluoride and Kryptofix 2.2.2

Anhydrous acetonitrile (5 ml) was added to a vial containing potassium fluoride (40 mg, 0.7 mmol) in water (0.5 ml) and Kryptofix 2.2.2 (200 mg, 0.54 mmol) in anhydrous acetonitrile (1 ml). The mixture was stirred and then dried by the pressure of an argon stream. Acetonitrile (5 ml) was added and dried again. The vial was cooled and **5** (150 mg, 0.22 mmol) in acetonitrile (5 ml) was added to the dried mixture. The vial was capped and heated at 40–60°C for 8 h. After cooling, the mixture was purified by preparative TLC on silica gel and eluted with chloroform–ethanol (9 : 1, v/v). The fluoride compound was separated ($R_f = 0.35$). The identity of the product was confirmed by comparison with an authentic sample of **6a** prepared previously.

Preparation of [^{18}F]cholesteryl 4-fluoromethyl benzoate 6b

Compound **6b** was prepared from **5** using a procedure similar to that used above for **6a**. The target solution (1.44 ml) was eluted with potassium carbonate solution (1%, 100 µl), containing [^{18}F]fluoride (6 mCi) of activity and transferred to a Reactivial^{TM} (2 ml, volume) containing Kryptofix 222 (10 mg, 0.027 mmol) and anhydrous acetonitrile (0.5 ml). The mixture was then evaporated to dryness by slight heat and the pressure of an argon stream. Drying was repeated after addition of two more portions of anhydrous acetonitrile (2×0.5 ml). A mixture of **5** (6 mg, 0.01 mmol) in Sure-Seal^{TM} grade acetonitrile (0.25 ml) was added to the above dried sample and heated at 40–60°C for 20 min. After cooling, the mixture was injected into the HPLC (Si Kromasil) instrument using a mixture of dichloromethane–acetonitrile–acetic acid (14 : 85 : 1) as the mobile phase (flow rate: 2 ml/min). The fraction eluted at 6.7 min was recovered. The active solution was checked for radiochemical purity by TLC on a polymer-backed silica gel layer eluted with chloroform ($R_f = 0.35$) using a Canberra spectroscopy unit armed with a step-motor. Radio thin layer chromatography showed a high purity (greater than 95%) in the form of [^{18}F]-CFMB. The final mixture was then dried by the pressure of an argon stream and slight heat prior to use in animal tests. The fraction eluted at 6.7 min was evaporated to dryness and reconstituted in borate buffer (0.2 ml, pH = 8) followed by passing through a 0.22 µm filter.

Table 1. Effect of ethynyl esteradiol injection on biodistribution of 6b in mature female mice 1 h after administration. Tissue uptake is represented as %1D/g ± SD with a dose between 30 ± 2 µCi ($n = 5$)

Pretreated test	Control	Tissue
21.7 ± 7.95	4.23 ± 0.34	Adrenal
10.2 ± 1.34	1.23 ± 0.16	Adrenal/blood
543 ± 167	36.1 ± 11.2	Adrenal/muscle
1.86 ± 0.51	3.26 ± 0.45	Ovaries
2.29 ± 0.55	4.94 ± 0.23	Blood
0.51 ± 0.07	0.49 ± 0.04	Bone
0.18 ± 0.05	0.19 ± 0.05	Fat
0.76 ± 0.11	0.77 ± 0.18	Kidney
7.14 ± 0.78	4.98 ± 0.18	Liver
0.04 ± 0.003	0.05 ± 0.01	Muscle
23.5 ± 3.32	0.98 ± 0.24	Spleen

Administration of [^{18}F]cholesteryl 4-(fluoromethyl) benzoate to mice

Mature female NMRI mice (Pasteur Institute, Iran) weighing 20–25 g ($n = 10$) were used in experiments. The animals were kept in groups of ten, in cages under constant temperature (24°C) and 12 h light/dark schedule. They had free access to standard mouse diet and water except during the experiment. On the day of the experiment, animals were randomly transferred to individual cages and allowed to acclimatize for 30 min before injection of radioligand. Ethynyl esteradiol was suspended in a mixture of Tween 80 solution (0.5%) and carboxymethyl cellulose solution (1%) and the mixture was agitated vigorously. The animals were divided into two groups. In the first group of ten mice, ethynyl esteradiol was injected subcutaneusly under their thigh skins 2 h before ^{18}F-CFMB injection. The second group of ten (control animals) was given only ^{18}F-CFMB as shown in Table 1.

The dried radioactive residue obtained in the last part was dissolved in borate buffer (0.5 ml, pH = 8) with agitation and warming for half a minute and injected to the mice intravenously.

Conclusion

Total synthesis and purification of the ^{18}F-labelled cholesterol derivative, [^{18}F]CFMB, took about 45 min with 75% yield (decay corrected) with a specific radioactivity of 2000–2200 Ci/mmol. Preliminary biodistribution studies on mice demonstrated that ^{18}F-CFMB is a good candidate for adrenal and ovaries tomography as a result of its good target to non-target ratio.

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