Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 13 February 2013, Revised 16 May 2013, Accepted 19 May 2013 Published online 9 July 2013 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3076

Alternative synthesis for the preparation of 16α-[18F]fluoroestradiol

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We have developed a new precursor, 3,17β-*O*-*bis*(methoxymethyl)-16β-*O*-*p*-nitrobenzenesulfonylestriol (14c) of 16α-[^{18}F] fluoroestradiol ([^{18}F]FES). Although we could not selectively protect the C17 alcohol in the presence of the C16 alcohol, we were able to prepare and chromatographically isolate the desired C16 TBDMS, C17,C3-dimethoxymethyl (diMOM) protected estriol derivative and convert into the ultimate fluorination precursor. The MOM protective group proved to be more quickly removed than the cyclic sulfate group. The di-MOM protective precursor at the C3 and C17 alcohols instead of a cyclic sulfate group shortened hydrolysis time. We prepared three different sulfonate precursors at C16 alcohol. After checking their reactivity in the [^{18}F]fluorination step and considering the stability of the precursors, we obtained the best results with nosylate precursor 14c.

Keywords: 16α-[^{18}F]Fluoroestradiol; [^{18}F]FES; imaging of estrogen receptor; PET; radiopharmaceutical

Introduction

16α-[^{18}F]Fluoroestradiol ([^{18}F]FES, Figure 1) is a promising positron emission tomography (PET) radiopharmaceutical for imaging estrogen receptors (ER) in breast cancer.[1–7] The identification of the ER status (positive or negative) is very important in treating breast cancers. In clinical practice, *in vitro* ER assays are usually used to predict tumor response to hormonal therapy and patient prognosis.[8–11] The high binding affinity of [^{18}F]FES for ER provides a mechanism for the selective accumulation within ER positive breast cancers. PET imaging of ER is a noninvasive alternative to *in vitro* assays.[4–6,12–14] In this aspect, the efficient preparation of [^{18}F]FES is very important. Two synthetic methods for 16α-[^{18}F]fluoroestradiol have been reported (Scheme 1).

16α-[^{18}F]Fluoroestradiol was first developed by the Katzenellenbogen group in 1984 from 3,16β-*bis*trifluoromethanesulfonate of 16β-hydroxyestrone precursor **2**.[15,16] After [^{18}F]fluorination, a reduction step using $LiAlH_4$ is required. In this reduction step, 17α-epimeric alcohol is produced as a byproduct and must be separated.[17–20] A second method was developed by Lim *et al*.[21] to preclude formation of 17-epimers using 3-methoxymethyl-l6β,17β-epistriol-*O*-cyclic sulfate (**3**). As this procedure does not lead to epimerization under radiolabeling conditions, this route is most commonly used among the several available precursors. Romer *et al*., Liang *et al*., Knott *et al*., and our group also reported manual syntheses and automatic production of [^{18}F]FES using the cyclic sulfate precursor **3**.[22–25] [^{18}F]FES was prepared in two steps – fluorination and acid hydrolysis of the sulfate. Although the fluorination step resulted in high radiolabeling yield, some difficulties were experienced in the acid hydrolysis by triple azeotropic distillation methods using 2 N HCl and acetonitrile. In closing reaction vial with 2 N HCl, the hydrolysis did not occur. It took more than 30 min by evaporation of solvent with triple azeotropic distillation in an open system to increase concentration of reagents. This hydrolysis method cannot be conveniently applied to an automatic module for routine production.[25] In this paper, we report a new synthetic pathway that is applicable to the automated production of [^{18}F]FES.

Experimental

General procedures

All chemicals were purchased from commercial suppliers (Sigma-Aldrich, TCI, Acros) and used without further purification. Analytical TLC was carried out on a pre-coated plate (Merck, silica gel 60 F_{254}). Flash column chromatography was performed with a silica gel (Merck, 230–400 mesh, ASTM). ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400-MR (400 MHz, Agilent Technologies, USA) spectrometer at ambient temperature.

*3,16β-O-Bis(benzyloxycarbonyl)estrone (**9**)*

16β-Hydroxyestrone[26] (**7**, 1.00 g, 3.49 mmol) was dissolved in dry THF (10 mL). TMEDA (0.63 mL, 4.20 mmol) was added, and the solution was cooled to 0 °C. Benzyl chloroformate (1.09 mL, 7.64 mmol) was added, and the cooled mixture was stirred for 2 h. The reaction was quenched

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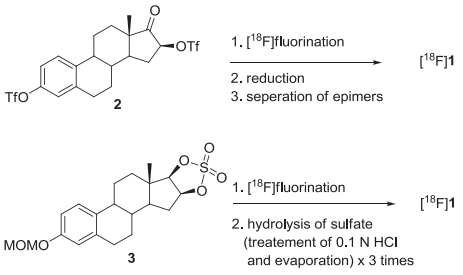


Scheme 1. Two synthetic methods for 16α-[^{18}F]fluoroestradiol.

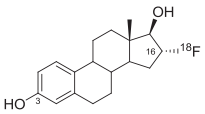


Figure 1. Structure of 16α-[^{18}F]fluoroestradiol ([^{18}F]FES, [^{18}F]**1**).

with 10 mL of water. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (20% ethyl acetate/hexane) afforded product **9** (1.6 g, 81%) as a foamy colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H), 1.43–1.87 (m, 6H), 1.93–2.05 (m, 2H), 2.35–2.49 (m, 3H), 2.85–2.92 (m, 2H), 4.92 (s, 1H), 5.22 (s, 2H), 5.25 (s, 2H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.33–7.46 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 25.6, 27.3, 29.3, 35.6, 36.2, 37.1, 41.8, 43.8, 44.1, 70.1, 70.2, 88.6, 118.2, 121.0, 126.1, 128.2, 128.4, 128.46, 128.52, 128.6, 134.7, 134.8, 137.1, 137.7, 149.0, 153.7, 154.7, 209.3; MS FAB$^+$ *m/z* 555.2 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{34}H_{35}O_7$ (M + H$^+$) 555.2383, found 555.2379.

*3-O-Benzyloxycarbonyl-16β,17β-estriol (**10**)*

3,16*β*-*O*-*Bis*(benzyloxycarbonyl)estrone (**9**, 100 mg, 0.18 mmol) was dissolved in dry THF (4 mL), followed by the addition of lithium tri-*t*-butoxyalumminium hydride (0.56 mL, 0.595 mmol) under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature. The reaction was quenched with ethyl acetate followed by the addition of 1N HCl (2.5 mL) and ice water (5 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The organic extracts were dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (25% ethyl acetate/hexane) afforded product **10** (50 mg, 66%) as a foamy colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H), 0.96–1.07 (m, 1H), 1.22–1.40 (m, 3H), 1.45–1.60 (m, 2H), 1.84–1.94 (m, 1H), 1.99 (dt, *J* = 12.4, 3.2 Hz, 1H) 2.35–2.17 (m, 3H), 2.58 (bs, 2H), 2.79–2.95 (m, 2H), 3.45 (d, *J* = 7.6 Hz, 1H), 4.20 (td, *J* = 12.6, 7.6 Hz, 1H), 5.25 (s, 2H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.92 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.32–7.46 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 26.1, 27.4, 29.7, 35.0, 37.5, 38.0, 43.0, 44.5, 46.7, 70.3, 70.5, 81.0, 118.3, 121.2, 126.6, 128.8, 128.9, 129.0, 135.0, 138.36, 138.44, 149.1, 154.2; MS (EI +) *m/z* 404 (M$^+$-H_2O). HRMS EI calcd for $C_{26}H_{28}O_4$ (M$^+$-H_2O) 404.1977, found 404.1988.

*3,17β-O-Bis(methoxymethyl)-16β-O-t-butyldimethylsilylestriol (**12a**)*

[27]3-*O*-Methoxymethyl-16*β*,17*β*-estriol (**4**, 1.59 g, 4.78 mmol), *N,N*-dimethylaminopyridine (0.580 g, 4.78 mmol), and *t*-butyldimethylsilyl chloride (0.870 g, 5.74 mmol) were dissolved in anhydrous dimethylformamide (DMF) (16 mL). Triethylamine (3.4 mL, 24.0 mmol) was added, and the reaction mixture was stirred for 24 h at room temperature. Ethyl acetate (40 mL) was added and the solution was washed with 1N HCl (2 × 50 mL) and saturated $NaHCO_3$ (50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (10% ethyl acetate/hexane) afforded **11a** with undesired byproduct **11b** (1.96 g, 92%, as a mixture of **11a** and **11b**). The mixture was dissolved in anhydrous DMF (20 mL), followed by the addition of *N,N*-diisopropylethylamine (3.96 mL, 23.9 mmol) and methoxymethyl chloride (1.82 mL, 23.9 mmol). The reaction mixture was heated at 60 °C for 5 h. Ethyl acetate (50 mL) was added, and the solution was washed with saturated $NaHCO_3$ (40 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (5% ethyl acetate/hexane) afforded products **12a** (1.2 g, 51% from **4**) and **12b** (0.51 g, 22% from **4**) as a yellowish oil. (**12a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.89 (s, 9H), 0.94 (s, 3H), 1.23–1.38 (m, 3H), 1.44–1.58 (m, 2H), 1.82–1.91 (m, 1H), 1.98 (dt, *J* = 12.4, 3.2 Hz, 1H), 2.14–2.32 (m, 4H), 2.77–2.93 (m, 2H), 3.34–3.42 (m, 1H), 3.40 (s, 3H), 3.47 (s, 3H), 4.24–4.30 (m, 1H), 4.67 (ABq, *J* = 6.6 Hz, 2H), 5.14 (s, 2H), 6.76 (d, *J* = 2.8 Hz, 1H), 6.82 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.7, −4.0, 13.1, 18.3, 26.1, 26.3, 27.6, 30.1, 37.1, 38.0, 38.3, 42.7, 44.5, 47.3, 55.4, 56.2, 69.5, 85.3, 94.7, 95.2, 113.9, 116.4, 126.5, 134.1, 138.1, 155.2; MS FAB$^+$ *m/z* 491.3 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{28}H_{47}O_5Si$ (M + H$^+$) 491.3193 (M + H$^+$), found, 491.3184. (**12b**): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.88 (s, 3H), 0.93 (s, 9H), 1.12–1.64 (m, 5H), 1.80–1.96 (m, 2H), 2.10–2.37 (m, 4H), 2.80–2.91 (m, 2H), 3.37 (s, 3H), 3.42–3.55 (m, 1H), 3.47 (s, 3H), 3.90–4.04 (m, 1H), 4.67 (s, 2H), 5.14 (s, 2H), 6.77 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ −4.4, −4.3, 12.2, 18.4, 26.0, 26.2, 27.4, 29.9, 34.0, 37.7, 38.3, 43.3, 44.3, 46.6, 55.6, 56.0, 76.8, 82.7, 94.5, 97.0, 113.8, 116.3, 126.4, 134.0, 138.1, 155.1; MS FAB$^+$ *m/z* 491.3 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{28}H_{47}O_5Si$ (M + H$^+$) 491.3193, found 491.3182.

*3,17β-O-Bis(methoxymethyl)-16β-estriol (**13**)*

3,17*β*-*O*-*Bis*(methoxymethyl)-16*β*-*O*-*t*-butyldimethylsilylestriol (**12a**, 0.640 g, 1.30 mmol) was dissolved in THF (5 mL). Tetrabutylammonium fluoride (TBAF) hydrate (0.44 g, 1.69 mmol) was added, and the reaction mixture was stirred for 18 h at 80 °C. Dichloromethane (20 mL) was added, and the solution was washed with water (20 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (30% ethyl acetate/hexane) afforded product **13** (0.48 g, 98%) as a yellowish oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 3H), 1.02–1.11 (m, 1H), 1.28–1.46 (m, 3H), 1.47–1.69 (m, 2H), 1.86–1.93 (m, 1H), 1.98 (dt, *J* = 12.0, 3.2 Hz, 1H), 2.16–2.24 (m, 1H), 2.25–2.32 (m, 2H), 2.75–2.81 (m, 1H), 2.82–2.90 (m,. 2H), 3.41 (d, *J* = 7.2 Hz, 1H), 3.44 (s, 3H), 4.47 (s, 3H), 4.18–4.24 (m, 1H), 4.75 (q, *J* = 6.4 Hz, 2H), 5.14 (s, 2H), 6.77 (d, *J* = 2.8 Hz, 1H), 6.83 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 26.3, 27.6, 30.0, 34.7, 37.9, 38.3, 42.9, 44.4, 47.2, 56.0, 56.2, 69.6, 87.6, 94.7, 97.2, 113.9, 116.4, 126.4, 133.9, 138.1, 155.2; MS FAB$^+$ *m/z* 377.2 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{22}H_{33}O_5$ (M + H$^+$) 377.2328; found 377.2318.

*3,17β-O-Bis(methoxymethyl)-16β-O-methanesulfonylestriol (**14a**)*

3,17*β*-*O*-*Bis*(methoxymethyl)-16*β*-estriol (**13**, 51 mg, 0.13 mmol) was dissolved in pyridine (0.5 mL) and cooled in an ice bath under an argon atmosphere. Methanesulfonyl anhydride (47 mg, 0.27 mmol) in pyridine (0.5 mL) was added, and the reaction mixture was stirred for 2 h at 0 °C. Ethyl acetate (10 mL) was added, and the solution was washed with 1N HCl (2 × 10 mL) and saturated $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (30% ethyl acetate/hexane) afforded product **14a** (58 mg, 95%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 3H), 1.03–1.12 (m, 1H), 1.28–1.42 (m, 2H), 1.47–1.58 (m, 2H), 1.70 (td, *J* = 13.6, 4.4 Hz, 1H), 1.81–1.89 (m, 1H), 1.99 (dt, *J* = 3.2, 2.4 Hz, 1H), 2.21 (td, *J* = 11.2, 4.4 Hz, 1H), 2.26–2.34 (m, 1H), 2.40–2.49 (m, 1H), 2.82–2.88 (m, 2H), 3.04 (s, 3H), 3.42 (s, 3H), 3.46 (s, 3H), 3.58 (d, *J* = 7.2 Hz, 1H), 4.71 (ABq, *J* = 6.6, Hz, 2H), 5.13 (s, 2H), 5.06–5.18 (m, 1H), 6.76 (d, *J* = 2.8 Hz, 1H), 6.82 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 26.2, 27.5, 29.9, 33.6, 37.4, 38.1, 39.0, 42.9, 44.3, 47.2, 56.0, 56.2, 78.7, 84.6, 94.7, 96.3, 114.0, 116.5, 126.4, 133.5, 137.9, 155.3; MS FAB$^+$ *m/z* 455.2 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{23}H_{35}O_7S$ (M + H$^+$) 455.2103; found 455.2117.

*3,17β-O-Bis(methoxymethyl)-16β-O-p-toluenesulfonylestriol (**14b**)*

3,17*β*-*O*-*Bis*(methoxymethyl)-16*β*-*O*-*p*-toluenesulfonylestriol was prepared by similar method for **14a**: 87% yield as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (s, 3H), 0.96–1.06 (m, 1H), 1.26–1.37 (m, 2H), 1.43–1.53 (m, 2H), 1.54–1.63 (m, 1H), 1.74–1.82 (m, 1H), 1.96 (dt, *J* = 12.0, 3.6 Hz, 1H), 2.13–2.33 (m, 3H), 2.45 (s, 3H), 2.79–2.87 (m, 2H), 3.33 (s, 3H), 3.46 (s,

3H), 3.51 (d, J = 7.2 Hz, 1H), 4.51 (s, 2H), 4.92–4.98 (m, 1H), 5.13 (s, 2H), 6.76 (d, J = 2.8 Hz, 1H), 6.82 (dd, J = 8.4, 2.8 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.6, 22.0, 26.2, 27.5, 29.9, 33.7, 37.3, 38.0, 42.9, 44.3, 47.2, 55.7, 56.2, 78.3, 78.4, 83.9, 94.7, 95.6, 104.0, 114.0, 116.4, 126.4, 127.9, 129.9, 133.6, 134.6, 137.9, 144.6, 155.3; MS FAB$^+$ *m/z* 531.2 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{29}H_{39}O_7S$ (M + H$^+$) 531.2416; found 531.2394.

*3,17β-O-Bis(methoxymethyl)-16β-O-p-nitrobenzenesulfonylestriol (**14c**)*

3,17β-*O*-*Bis*(methoxymethyl)-16β-*O*-*p*-nitrobenzenesulfonylestriol was prepared by similar method for **14a**: 94% as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.99–1.08 (m, 1H), 1.27–1.38 (m, 2H), 1.43–1.53 (m, 2H), 1.54–1.64 (m, 1H), 1.74–1.82 (m, 1H), 1.96 (dt, J = 12.4, 3.6 Hz, 1H), 2.17 (td, J = 11.6, 4.4 Hz, 1H), 2.26–2.36 (m, 2H), 2.78–2.86 (m, 2H), 3.33 (s, 3H), 3.46 (s, 3H), 3.54 (d, J = 7.2 Hz, 1H), 4.54 (ABq, J = 6.4 Hz, 2H), 5.07–5.18 (m, 1H), 5.13 (s, 2H), 6.75 (d, J = 2.4 Hz, 1H), 6.81 (dd, J = 8.8, 2.4 Hz, 1H), 7.16 (d, J = 8.8, 1H), 8.12 (d, J = 8.8 Hz, 2H), 8.38 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.5, 26.0, 27.4, 29.7, 33.4, 37.2, 37.9, 42.8, 44.2, 47.1, 55.8, 56.1, 80.5, 84.2, 94.7, 96.0, 114.1, 116.5, 124.5, 126.5, 129.2, 133.5, 137.9, 143.6, 150.7, 155.4; MS FAB$^+$ *m/z* 562.2 (M + H$^+$). HRMS FAB$^+$ calcd for $C_{28}H_{36}O_9NS$ (M + H$^+$) 562.2111; found 562.2087.

*16α-Fluoroestradiol (**1**)*

3,17β-*O*-*Bis*(methoxymethyl)-16β-*O*-*p*-nitrobenzenesulfonylestriol **14c** (11 mg, 0.02 mmol) was dissolved in acetonitrile (0.1 mL) followed by the addition of *tert*-amyl alcohol (0.5 mL) and *n*-tetrabutylammonium fluoride (26 mg, 0.1 mmol). The reaction mixture was stirred for 1 h at 120 °C. The reaction solvents were evaporated to dryness *in vacuo*. Aqueous hydrochloric acid (2N, 1 mL) was added, and the reaction mixture was heated at 120 °C for 15 min. Dichloromethane (2 mL) was added, and the solution was washed with water (2 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by flash column chromatography (3% methanol/dichloromethane) afforded product **1** (3.0 mg, 52%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H), 1.34–1.49 (m, 2H), 1.58 (s, 2H), 1.60–1.68 (m, 1H), 1.81–1.97 (m, 5H), 2.20–2.34 (m, 2H), 2.79–2.86 (m, 2H), 3.86 (d, J = 28.8 Hz, 1H), 4.68 (s, 1H), 4.86–4.93 and 5.00–5.06 (dm, J = 52.0 Hz, 1H), 6.56 (d, J = 2.4 Hz, 1H), 6.63 (dd, J = 8.4, 2.8 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 13.0, 27.3, 28.7, 30.8, 32.8 (d, J = 23.5 Hz, C15), 38.0, 40.0, 45.3, 45.4, 49.2, 88.6 (d, J = 22.0 Hz, C17), 101.5 (d, J = 177.3 Hz, C16-F), 113.8, 116.1, 127.1, 132.3, 138.7, 156.0; Registry No. 92817-10-2.

16α-[^{18}F]Fluoroestradiol

[^{18}F]F$^-$/H$_2^{18}$O was trapped on a Chromafix PS-HCO$_3$ cartridge (Machery-Nagel, Germany), and [^{18}F]F$^-$ was eluted with 600 μL of stock solution (0.1 M potassium mesylate (KOMs) in H_2O (250 μL) and 22 mg of K_{222} in methanol (1.0 mL)). The radioactivity was dried with 1 mL × 3 times of acetonitrile by the azeotropic-distillation method. After complete drying, the nosylate precursor (**14c**, 2 mg, 3.67 μmol) in 100 μL of acetonitrile and 500 μL of *t*-amyl alcohol were added to the reactor vial. [^{18}F]Fluorination was carried out at 120 °C for 20 min. The reaction mixture was evaporated and dissolved in acetonitrile (100 μL). Aqueous hydrochloric acid (1N, 500 μL) was added to the reactor vial, and then the reactor was heated at 120 °C for 3 min for hydrolysis. After cooling, aqueous sodium hydroxide (2N, 250 μL) was added for neutralization, and 200 μL of citrate buffer was added. This solution was injected into the high-performance liquid chromatography (HPLC) system for purification. HPLC purification was conducted with a Nucleosil 100-7 C18 250 × 16 semi-prep column (Macherey-Nagel) eluted at 5 mL/min with 30% EtOH:40% water:30% acetonitrile. Purified 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) was eluted. We obtained a decay-corrected radiochemical yield (RCY) of 19–24% (n = 3) after HPLC purification for manual synthesis. Total synthesis time, including purification time, was about 36 min.

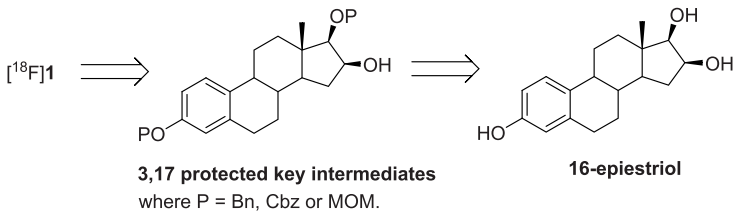
Results and discussion

Although we have used the second synthetic route from cyclic sulfate precursor **3** using an automatic module, we faced two difficulties in routine production. One is an HPLC separation problem, and the other is an increase of preparation time from three repeated processes in the hydrolysis step of the sulfate. These two difficulties motivated us to develop a new preparation route of [^{18}F]FES. The basic idea of the new strategy is shown as a retrosynthetic route in Scheme 2. If we can selectively protect C3 and C17β-alcohol groups from 16β-epistriol, we can activate the 16β-alcohol group by suitable leaving group such as sulfonate to introduce [^{18}F]fluoride. The protective groups on C3 and C17β should be easily removed after the labeling step. We tried to introduce a more convenient protective group on C17 alcohol. As protective groups, benzyl, benzylcarbonate (Cbz), and methoxymethyl (MOM) groups were chosen.

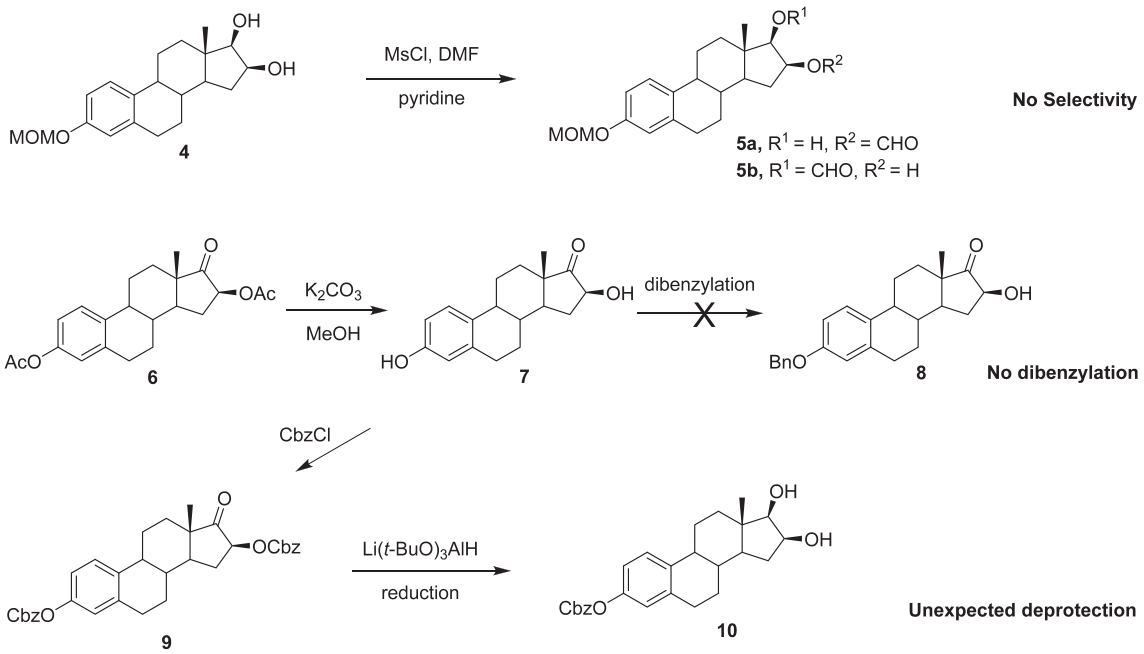
Synthetic trials of the selective protection of C16 alcohol or C17 alcohol

Our first attempt to synthesize selective protective estriol of either C16 alcohol or C17 alcohol of 16β-epistriol is shown in Scheme 3. Stein *et al.* reported a selective formyl protecting method of C16 alcohol in dl-13-ethylgona-1,3,5(10)-triene-3,16β,17β-triol under a USA patent in 1972.[28] We planned to introduce the selective formylation of C16 alcohol and a MOM protecting group of C17β alcohol in 3-*O*-methoxymethyl-16β,17β-estriol (**4**). Unfortunately, the formylation reaction of the diol **4** gave a 1:1 mixture of formylated products of C16β alcohol and C17β alcohol, which was confirmed by NMR spectroscopy.

To make 3-*O*-protected-16β-protected-17β-estriol, we also attempted to protect C16β alcohol from 3-*O*-protected-16β-hydroxyestrone, followed by reduction of the ketone group. The starting material, 3-*O*-protected-16β-hydroxyestrone, was prepared from 3,16β-dihydroxyestrone (**7**), which was obtained from 3,16β-*O*-diacetylestrone (**6**) by treatment with K_2CO_3 in good yield.[27] Dibenzylation of 3,16β-dihydroxyestrone (**7**) was carried out with excess amounts (10 equiv) of benzyl bromide and K_2CO_3 in acetone at 50 °C overnight. Unfortunately, we obtained only monobenzylation product **8** of C3 alcohol in 3,16β-dihydroxyestrone (**7**). This result is most likely because of low reactivity of the secondary C16β alcohol position. To



Scheme 2. Retrosynthetic route.

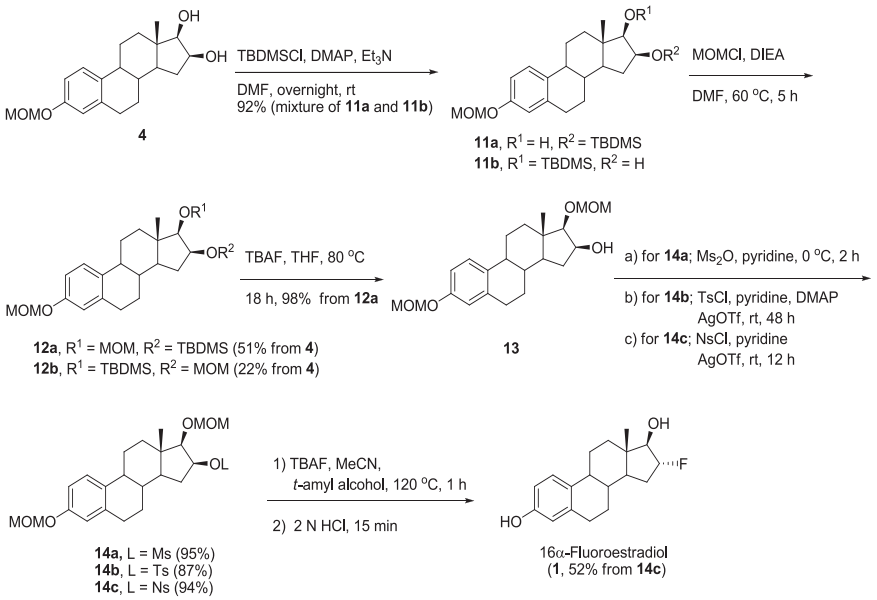


Scheme 3. Failed reactions of selective protection at C17 position.

overcome the low reactivity of the benzyl group (alkylation reaction), we introduced the Cbz group (acylation reaction). This group could also readily be removed by hydrogenation using Pd/C. 3,16β-Dibenzyloxycarbonylestrone (di-Cbz-estrone, **9**) was prepared in 81% yield from 3,16β-dihydroxyestrone (**7**) and benzyl chloroformate (2.2 equiv). Reduction of the C17 keto group of di-Cbz-estrone (**9**) with lithium tri-*t*-butoxy-aluminum hydride (Li(*t*-BuO)$_3$AlH) provided 3-*O*-benzyloxycarbonyl-16β,17β-estriol (**10**), an unexpected product. Generally, the Cbz protected functionality is stable under the Li(*t*-BuO)$_3$AlH hydride reaction conditions.[29]

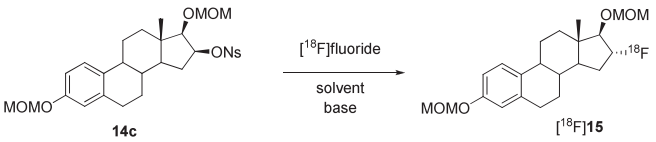
Laurent *et al.* reported a selective silyl ether protection method of C16 alcohol in 1,3,5(10)-estratriene-3,16α,17β-diol (estriol) in 1987.[30] We conducted the selective silylation reaction using various bulky silyl ethers. 3-*O*-Methoxymethyl-16β-*O*-*t*-butyldiphenylsilylestriol was prepared from 3-*O*-methoxymethyl-16β,17β-estriol (**4**) and *t*-butyldiphenylsilyl chloride (TBDPSCl) in good yield. Unfortunately, the 17β-methoxymethyl derivative of 3-*O*-methoxymethyl-16β-*O*-*t*-butyldiphenylsilylestriol could not be prepared from 3-*O*-methoxymethyl-16β-*t*-butyldiphenylsilylestriol. The 17β alcohol was located in the steric hindered position, and hence, alkylation at 17β alcohol using MOM chloride did not proceed well.

We also tried the selective silylation reaction using the *t*-butyldimethylsilyl (TBDMS) group, which is less bulky than the TBDPS group. The introduction of the TBDMS group could be



Scheme 4. Optimized synthetic route for 16α-fluoroestradiol (**1**).

Table 1. [^{18}F]Fluorination using the FES nosylate precursor **14c** under various conditions



Entry	Solvent(s)[a]	Base	Base amount (μmol)	R-TLC yield (%)[b] 10 min	R-TLC yield (%)[b] 20 min	^{18}F in solution (%) at 20 min[c]	RCY (%)
1	*t*-amyl alcohol	TBAOH	4 μL (6)	31.3	51.4	80.0	41.1
2	*t*-amyl alcohol	TBAOH	8 μL (12)	17.7	21.9	92.1	20.2
3	*t*-amyl alcohol	CsOH	1.3 μL (6)	9.9	16.7	90.9	15.2
4	*t*-amyl alcohol	CsOH	2.6 μL (12)	0.5	0.8	91.8	0.7
5	*t*-amyl alcohol	KOH	0.35 mg (6)	37.1	50.3	84.7	42.6
6	*t*-amyl alcohol	KOH	0.70 mg (12)	14.8	15.8	95.3	15.1
7	*t*-amyl alcohol	$TBAHCO_3$	4.7 μL (6)	21.7	25.8	94.7	24.4
8	*t*-amyl alcohol	$TBAHCO_3$	9.4 μL (12)	4.8	5.8	96.6	5.6
9	*t*-amyl alcohol	$KHCO_3$	0.62 mg (6)	28.1	28.0	95.0	26.2
10	*t*-amyl alcohol	$KHCO_3$	1.24 mg (12)	10.4	10.7	95.8	10.3
11	*t*-amyl alcohol	K_2CO_3	0.85 mg (6)	22.3	23.1	95.4	22.0
12	*t*-amyl alcohol	K_2CO_3	1.70 mg (12)	1.3	0	96.6	0.0
13	acetonitrile	TBAOH	4 μL (6)	47.6	45.3	60.4	27.4
14	acetonitrile	KOH	0.35 mg (6)	26.5	32.0	78.2	25.0
15	*t*-amyl alcohol + acetonitrile[c]	TBAOH	2 μL (3)	25.4	34.5	66.7	23.0
16	*t*-amyl alcohol	TBAOH	2 μL (3)	23.4	56.5	78.0	44.1

FES, fluoroestradiol; RCY, radiochemical yield.
[a]Nosylate precursor **14c** (2 mg) in acetonitrile (100 μL) was added to the reactor, and then *t*-amyl alcohol (500 μL) was added.
[b]Radio-TLC yield, 30% ethyl acetate/hexane elution.
[c]*t*-Amyl alcohol (300 μL) and acetonitrile (300 μL) were used.

accomplished by the same method used to introduce the TBDPS group. Although the silylated product was formed in high yield (92%) and shown as a single spot on TLC, it proved to be a mixture of regioisomers based on ^{1}H NMR, and HPLC analysis data (**11a**:**11b** = 7:3). These mixtures could not be separated by normal phase silica gel chromatography. However, we found that MOM-protected **12a** and **12b** derivatives, prepared from a mixture of **11a** and **11b**, could be separated by silica gel column chromatography to provide **12a** and **12b** in 51% and 22% yield, respectively, from **4**. After the preparation of 3,17β-*O-bis* (methoxymethyl)-16β-*O*-*t*-butyldimethylsilylestriol (**12a**), the 16β-TBDMS group was removed by treatment of TBAF in refluxing THF for 18 h.

3,17β-*O-Bis*(methoxymethyl)-16β-estriol (**13**) was synthesized as a key intermediate. From this intermediate, three precursors can be synthesized with methanesulfonyl anhydride (for **14a**) or *p*-toluenesulfonyl chloride (for **14b**) or *p*-nitrobenzenesulfonyl chloride (for **14c**).

Using the nosylate precursor **14c**, we tested the fluorination step and hydrolysis reaction step using fluorine-19. The cold and hot fluorination adapted our fluorination method[31] using protic solvents to the new FES precursor. Among the three precursors, as we expect, the nosylate precursor gave best result. As it is quite stable at a room temperature for more than a month, we optimized fluorination reaction using nosylate precursor. The nosylate precursor **14c** was reacted with TBAF in acetonitrile and *t*-amyl alcohol. After removal of the solvents, the resulting MOM-protected fluoroestradiol was hydrolyzed under acidic conditions (2N HCl) to give the non-radioactive 16α-fluoroestradiol (**1**). Scheme 4 is an optimized synthetic route for 16α-fluoroestradiol (**1**).

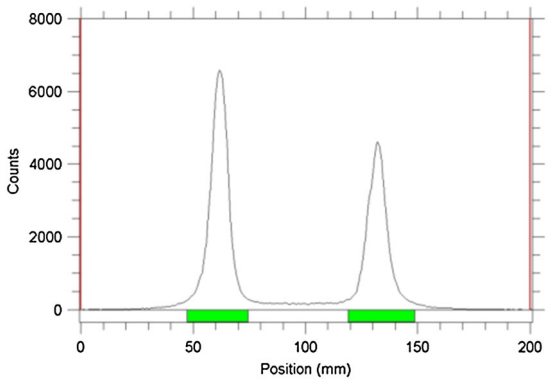
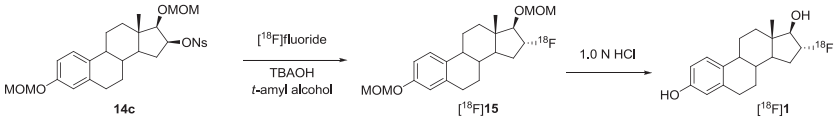


Figure 2. Typical radio thin-layer chromatography chromatogram of [^{18}F]**15** using 30% ethyl acetate/hexane as an elution solvent.



Scheme 5. Synthesis of [^{18}F]FES using Nosylate precursor **14c**.

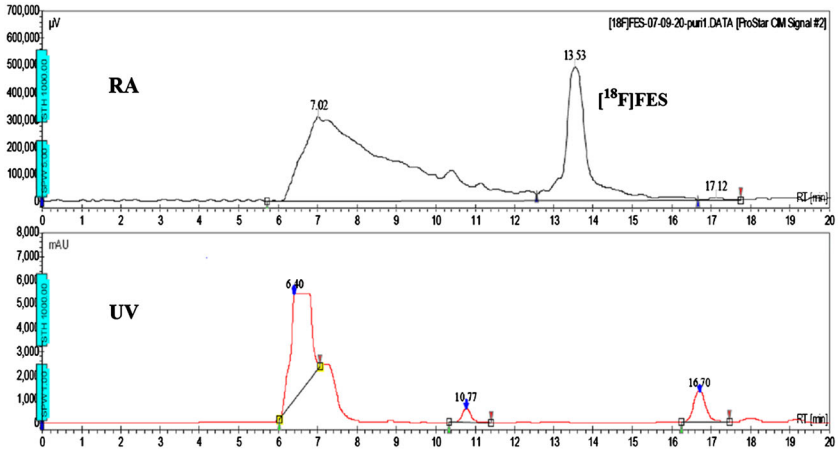


Figure 3. High-performance liquid chromatography chromatogram of 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) at the end of manual synthesis. The upper radioactive (RA) chromatogram shows a simple peak at retention times of 13–14 min corresponding to 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) with a Nucleosil 100-7 C18 250 × 16 mm semi-prep column (Macherey-Nagel, Germany) eluted at 5 mL/min with 30% EtOH:40% water:30% acetonitrile.

Optimization of the [^{18}F]fluorination conditions

Generally, an aliphatic nucleophilic substitution (S_N2) reaction is used with aprotic polar solvents such as acetonitrile, DMF, and dimethyl sulfoxide. Recently, our group developed an efficient fluorination method for the aliphatic S_N2 reaction with various tertiary alcohol solvents.[31] In this novel synthetic method system, the polar protic solvent enhances the nucleophilicity of the fluorine ions. We found a dramatic increase of the RCY for the [^{18}F]fluorination reaction. In this report, we developed a new nosylate precursor **14c** using a *t*-amyl alcohol solvent system for the [^{18}F]fluorination reaction.

Table 1 shows the results of the [^{18}F]fluorination using the nosylate precursor **14c** with various bases, in different amounts of base and in reaction solvents. To optimize the [^{18}F]fluorination conditions, several factors should be considered. We fixed the following reaction conditions: precursor amount, 2 mg/3.67 μmol; reaction temperature, 120 °C; reaction volume, 600 μL. [^{18}F] Fluorination yield was determined by radio thin-layer chromatography (radio-TLC). [^{18}F]Fluorination was monitored by radio-TLC at 10 min and 20 min. After 20 min of reaction, we obtained the results by radio-TLC yield after 20 min to determine the incorporation of [^{18}F]fluoride as well as by calibration of the activity in the vial.

To optimize [^{18}F]fluorination with various reaction conditions, [^{18}F]fluorination was performed as follows: [^{18}F]F^-/$H_2^{18}O$ (85.1–125.8 MBq) was trapped in a Chromafix PS-HCO_3 cartridge (Machery-Nagel, Germany), and [^{18}F]fluoride was eluted with 600 μL of stock solution (0.1 M KOMs in H_2O (250 μL) and 22 mg of K_{222} in methanol (1.0 mL)). We added additional base (e.g., TBAOH, CsOH, KOH, $TBAHCO_3$, $KHCO_3$ or K_2CO_3 with 3, 6, or 12 μmol) into the reactor vial to test the base effect. The radioactivity was then dried with 1 mL × 3 times of acetonitrile with heating under a stream of nitrogen. After drying, 2 mg (3.67 μmol) of FES nosylate precursor **14c** in 100 μL of acetonitrile and 500 μL of *t*-amyl alcohol were added to the reactor vial. Also, acetonitrile and 50% acetonitrile in *t*-amyl alcohol as a mixing reaction solvent was tested to check the solvent effect. A crude radio-TLC chromatogram of the reaction mixture is shown in Figure 2.

[^{18}F]Fluorination was first performed according to a previously reported labeling method.[31] RCY was measured by multiplying the relative integration of a radio TLC scan and the percentage of the radioactivity in the reaction solution. For [^{18}F]fluorination, 100 μL of acetonitrile and 500 μL of *t*-amyl alcohol were used as solvents with 4 μL of TBAOH (6 μmol) as an additional base, giving 41% of RCY (entry 1). When the amount of base (12 μmol) was increased, the yield was lower (entry 2). We found that the optimal amount of TBAOH was 2 μL (3 μmol, entry 16) to 4 μL (6 μmol). We also checked other kinds of bases (e.g., CsOH, KOH, $TBAHCO_3$, $KHCO_3$ and K_2CO_3). However, we could not obtain good results except for KOH (entry 5). From a comparison of solvents in [^{18}F] fluorination using 4 μL of TBAOH (6 μmol, entry 1 vs 13) and 0.35 mg of KOH (6 μmol, entry 5 vs 14), we obtained higher yields in the mixture of acetonitrile (100 μL) and *t*-amyl alcohol (500 μL).

Preparation of 16α-[^{18}F]fluoroestradiol ([^{18}F]1)

[^{18}F]Fluorination was carried out using 2 mg of nosylate precursor **14c** and [^{18}F]fluoride (7.4 GBq) in 100 μL acetonitrile and 500 μL of *t*-amyl alcohol at 120 °C for 20 min, as shown in Scheme 5. The reaction solvent was evaporated, and the residue was then dissolved in acetonitrile (100 μL). Aqueous hydrochloric acid (1N, 500 μL) was added to the reactor vial and then heated at 120 °C for 3 min for hydrolysis. After cooling the reaction mixture, aqueous sodium hydroxide (2N, 250 μL) was added for neutralization, followed by citrate buffer (200 μL). This solution was injected onto an HPLC system for purification. HPLC purification was conducted with a Nucleosil 100-7 C18 250 × 16 mm semi-prep column (Macherey-Nagel, Germany) eluted

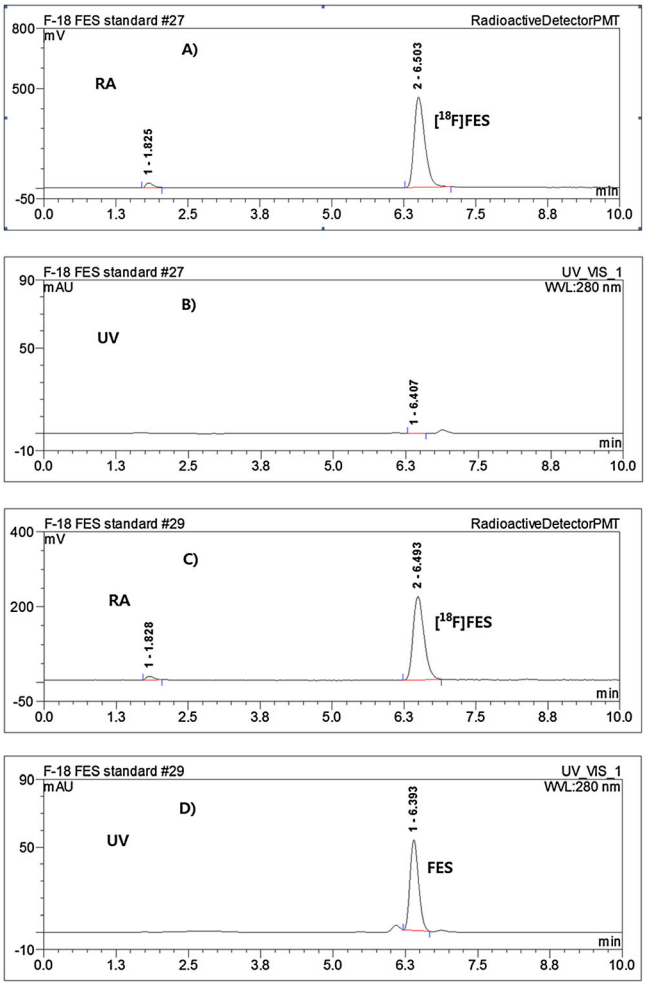


Figure 4. High-performance liquid chromatography chromatogram of purified 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) with a Luna C18(2) 250 × 4.6 mm, 1 mL/min, water/MeCN = 40:60, 280 nm: (A) and (B) Radio and UV chromatogram of quality control of purified [^{18}F]**1**; (C) and (D) radio and UV chromatogram of purified [^{18}F]**1** with coinjection of a cold authentic sample.

at 5 mL/min with 30% EtOH:40% water:30% acetonitrile. In the hydrolysis step, we could not find product-like impurities in the HPLC chromatogram (Figure 3). Collected 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) was analyzed by injection to HPLC (Luna C18(2) 250 × 4.6 mm, 1 mL/min, water/MeCN = 40:60, 280 nm), and the obtained HPLC chromatogram is shown in Figure 4. We obtained purified 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) with decay-corrected RCY of 19-24% (n = 3) after HPLC purification for manual synthesis. Total synthesis time including purification time was about 40 min. The specific activity of [^{18}F]**1** was 84.2 GBq/μmol.

Conclusion

We described a new synthesis of 16α-[^{18}F]fluoroestradiol ([^{18}F]**1**) using nosylate precursor **14c** as an alternative precursor. An efficient synthetic route for the preparation of nosylate precursor **14c** was also achieved. Using this nosylate precursor **14c**, [^{18}F]fluorination and hydrolysis steps are suitable for an automatic chemistry module. We also evaluated the effects of various bases and solvents on [^{18}F]fluorination with the use of the new precursor. We found that *t*-amyl alcohol showed higher yield than acetonitrile, and use of TBAOH (3 μmol) base was the best condition.

Conflict of Interest

The authors did not report any conflict of interest.

Acknowledgments

This research was supported by the Converging Research Program (2012 K001486) through the Ministry of Education, Science and Technology. High resolution mass spectra were carried out at the Korea Basic Science Institute (Daegu, Korea).

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