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Optimization of the alkyl side chain length of fluorine-18-labeled 7α-alkyl-fluoroestradiol

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ABSTRACT

Introduction: Several lines of evidence suggest that 7α-substituted estradiol derivatives bind to the estrogen receptor (ER). In line with this hypothesis, we designed and synthesized ^{18}F-labeled 7α-fluoroalkylestradiol (Cn-7α-[^{18}F]FES) derivatives as molecular probes for visualizing ERs. Previously, we successfully synthesized 7α-(3-[^{18}F]fluoropropyl)estradiol (C3-7α-[^{18}F]FES) and showed promising results for quantification of ER density *in vivo*, although extensive metabolism was observed in rodents. Therefore, optimization of the alkyl side chain length is needed to obtain suitable radioligands based on Cn-7α-substituted estradiol pharmacophores.

Methods: We synthesized fluoromethyl (**23**; C1-7α-[^{18}F]FES) to fluorohexyl (**26**; C6-7α-[^{18}F]FES) derivatives, except fluoropropyl (C3-7α-[^{18}F]FES) and fluoropentyl derivatives (C5-7α-[^{18}F]FES), which have been previously synthesized. *In vitro* binding to the α-subtype (ERα) isoform of ERs and *in vivo* biodistribution studies in mature female mice were carried out.

Results: The *in vitro* IC_{50} value of Cn-7α-FES tended to gradually decrease depending on the alkyl side chain length. C1-7α-[^{18}F]FES (**23**) showed the highest uptake in ER-rich tissues such as the uterus. Uterus uptake also gradually decreased depending on the alkyl side chain length. As a result, *in vivo* uterus uptake reflected the *in vitro* ERα affinity of each compound. Bone uptake, which indicates de-fluorination, was marked in 7α-(2-[^{18}F]fluoroethyl)estradiol (C2-7α-[^{18}F]FES) (**24**) and 7α-(4-[^{18}F]fluorobutyl)estradiol (C4-7α-[^{18}F]FES) (**25**) derivatives. However, C1-7α-[^{18}F]FES (**23**) and C6-7α-[^{18}F]FES (**26**) showed limited uptake in bone. As a result, *in vivo* bone uptake (de-fluorination) showed a bell-shaped pattern, depending on the alkyl side chain length. C1-7α-[^{18}F]FES (**23**) showed the same levels of uptake in uterus and bone compared with those of 16α-[^{18}F]fluoro-17β-estradiol.

Conclusions: The optimal alkyl side chain length of ^{18}F-labeled 7α-fluoroalkylestradiol was the shortest: C1-7α-[^{18}F]FES. Our results indicate that shorter chain lengths within the 4-Å ligand binding cavities of ERα are suitable for 7α-fluoroalkylestradiol derivatives.

1. Introduction

Fluorine-18-labeled steroid hormones are useful probes for positron emission tomography visualization of receptor-positive tumors such as breast and prostate cancer. Over the past 30 years, several derivatives of ^{18}F-labeled 17β-estradiol have been synthesized and evaluated. Among them, 16α-[^{18}F]fluoro-17β-estradiol (16α-[^{18}F]FES) [1] is currently used as the standard radioligand for imaging both primary and metastatic estrogen receptor-positive tumors [2,3].

On the other hand, several lines of evidence suggest that 7α-substituted estradiol derivatives bind to the estrogen receptor (ER), even though they have a long chain with complex functionality [4–11]. In contrast, small polar groups at the 7α-position do not bind well [12,13]. Furthermore, substitutions with 7α-alkyl chains bearing alcohol, carboxylic acid, and ester groups also have low affinity [6]. Thus, Anstead et al. recommended that groups at the 7α-position bind well to ERs, even if they are rather long; however, polar functions must be positioned away from the core of the steroid structure [14].

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In line with this hypothesis, we are interested in the design and synthesis of 7α-fluoroalkylestradiol (Cn-7α-[^{18}F]FES) as a molecular probe to visualize ER function. Previously, French et al. synthesized the five-carbon derivative 7α-(5-[^{18}F]fluoropentyl)estradiol (C5-7α-[^{18}F]FES) and evaluated its biodistribution in immature rats [5]. Although C5-7α-[^{18}F]FES showed somewhat selective uptake in target tissues, the levels of uptake into non-target tissues were high, possibly due to the increased lipophilicity of the additional five-carbon chain. In a recent follow-up study, we investigated the shorter three-carbon derivative 7α-(3-[^{18}F]fluoropropyl)estradiol (C3-7α-[^{18}F]FES) and characterized its biological properties [15]. As expected, the shorter three-carbon chain resulted in lower uptake into non-target tissues, such as fat and blood, and the uterus-to-blood ratio at 60 min was double that of the five-carbon chain derivative. However, the three-carbon derivative underwent greater metabolic de-fluorination than the five-carbon derivative. These results indicate that opportunities still exist for further optimization of the alkyl side chain length of Cn-7α-[^{18}F]FES derivatives.

Currently, the most common route for preparing 16α-[^{18}F]FES uses 3-methoxymethyl-16β,17β-epistriol-*O*-cyclic sulfate [16]. However, some difficulties remain regarding the time required for (>30 min) acid hydrolysis of the bisulphate intermediate [17], and further optimization is required for routine clinical use in individual facilities [18]. In contrast, di-methoxymethyl-protected groups of labeling precursors of Cn-7α-[^{18}F]FES derivatives may be removed more quickly and have acceptable times required for radiosynthesis.

In this study, we further synthesized fluoromethyl (**22**; C1-7α-[^{18}F]FES) to fluorohexyl (**25**; C6-7α-[^{18}F]FES) derivatives of Cn-7α-[^{18}F]FES, except fluoropropyl (C3-7α-[^{18}F]FES) and fluoropentyl derivatives (C5-7α-[^{18}F]FES), which were synthesized previously [5,15]. We characterized the *in vitro* binding and *in vivo* distribution of these derivatives in mature female mice compared to the previously published data for C3-7α-[^{18}F]FES and 16α-[^{18}F]FES, and we discuss the optimization of the alkyl side chain length.

2. Materials and methods

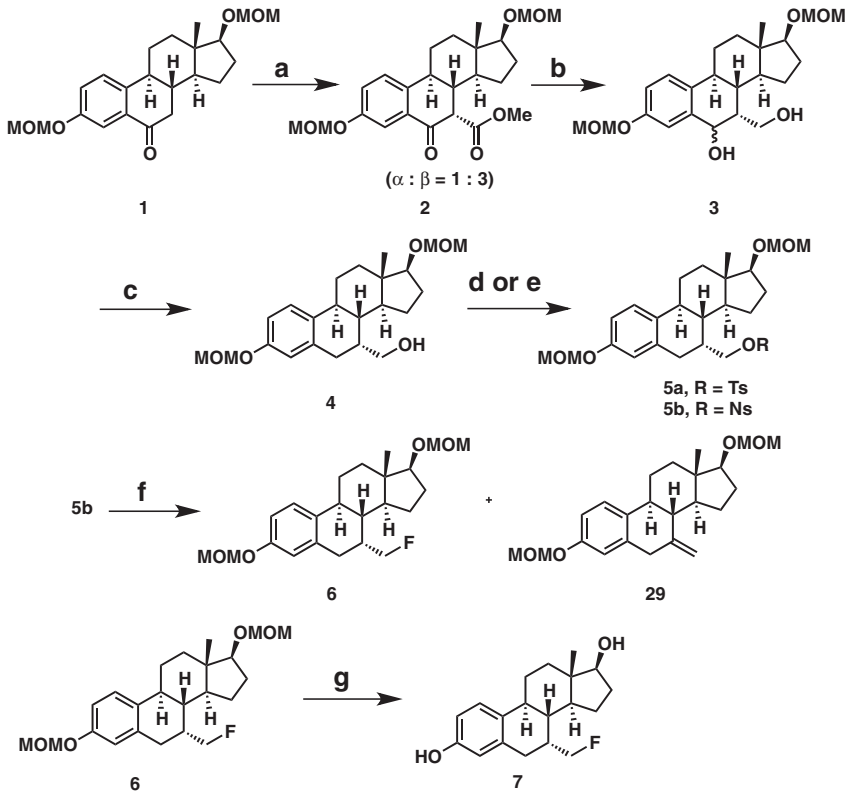
2.1. Chemical synthesis

The methods of synthesis of non-radioactive compounds are outlined in Schemes 1–3, and the details are summarized in the supporting information in the online version at http://dx.doi.org/10.1016/j.nucmedbio.2016.05.008.

2.2. Radiochemical synthesis

[^{18}F]Fluoride was produced by proton irradiation of ^{18}O-enriched water (Taiyo Nippon Sanso, Tokyo, Japan) at 50 μA for 5 min using the HM-20 cyclotron (Sumitomo Heavy Industries, Tokyo, Japan). Isolation of [^{18}F]fluoride from enriched water and subsequent ^{18}F-fluorination, de-protection, purification, and formulation were carried out automatically by using a multi-purpose synthetic apparatus (MPS-200; Sumitomo Heavy Industries). Preparative high-performance liquid chromatography (HPLC) was done using a Prominence (Shimadzu, Kyoto, Japan) that was equipped with an ultraviolet (UV) absorbance detector set at 280 nm and a semiconductor radiation detector system in the indicated conditions. Radiochemical purity and specific activity (SA) of the labeled compounds were determined with the same HPLC system using the indicated conditions.

[^{18}F]Fluoride was separated with anion exchange resin (Sep-Pak Light Accell Plus QMA; Nihon Waters, Tokyo, Japan). Elution with



Scheme 1. Synthesis schemes for the precursor for C1-7α-[^{18}F]FES (**5a** and **5b**) synthesis and authentic standard (**7**). Reagents and conditions: (a) sodium hydride, dimethyl carbonate, reflux, 1 h (yield: 24%; α:β = 1:3); (b) lithium aluminium hydride, tetrahydrofuran, room temperature, 3 h (yield: 87%); (c) hydrogen (1 atm), palladium hydroxide, ethanol, room temperature, 6 h (yield: 73%); (d) *p*-toluenesulfonyl chloride, triethylamine, dichloromethane, room temperature, overnight (yield of **5a**: 93%), (e) *p*-nitrobenzenesulfonyl chloride, triethylamine, dichloromethane, room temperature, 2 h (yield of **5b**: 73%); (f) tetra-*n*-butyl ammonium fluoride, *tert*-butyl alcohol, reflux, 20 min (yield of **6**: 14%, yield of **29**: 22%); (g) 1 N hydrochloric acid, tetrahydrofuran, reflux, 30 min (yield: 93%).