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Synthesis of 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol as a new potential PET agent for imaging of steroid sulfatase (STS) in cancers

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ABSTRACT

Steroid sulfatase (STS) catalyzes the hydrolysis of steroid sulfates to estrones, the main source of estrogens in tumors. Carbonic anhydrase II (CAII) is highly expressed in red blood cells through a coordination of the monoanionic form of the sulfamate moiety to the zinc atom in the enzyme active site, and CAII is highly expressed in several tumors. 2-Methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol (**5**) is a dual-function STS-CAII inhibitor inhibited STS with 39 nM IC_{50} value selectively over CAII with 379 nM IC_{50} value. This compound exhibited potent antiproferative activity with mean graph midpoint value of 87 nM in the NCI 60-cell-line panel, and antiangiogenic in vitro and in vivo activity in an early-stage Lewis lung model as well. The compound has been recently developed as a multitargeted anticancer agent. Both STS and CAII are over-expressed in cancers and have become attractive targets for cancer treatment and molecular imaging of cancer. Here we report the first design and synthesis of 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol ([^{11}C]**5**) as a new potential imaging agent for biomedical imaging technique positron emission tomography (PET) to image STS in cancers. The authentic standard **5** was synthesized from 17β-estradiol by published procedures in 5 steps with 40% overall chemical yield. The precursor 2-hydroxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol (**14a**) for radiolabeling was synthesized from 17β-estradiol in 10 steps with 5% overall chemical yield. The target tracer [^{11}C]**5** was prepared from the precursor **14a** with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) purification in 40–50% radiochemical yields based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 370–740 GBq/μmol specific activity at EOB.

1. Introduction

The enzyme steroid sulfatase (STS) catalyzes the hydrolysis of steroid sulfates to estrones, the main source of estrogens in tumors [1]. The enzyme carbonic anhydrase (CA) catalyzes the production of acid in tumors, and so far more than 10 enzymatically active CA isoenzymes have been identified [2]. CAII is highly expressed in several tumors, and is highly expressed in red blood cells through a coordination of the monoanionic form of the sulfamate moiety to the zinc atom in the enzyme active site [3]. Both STS and CAII are over-expressed in cancers and have become attractive clinical targets for the treatment of cancers [1–3], and dual-function STS-CAII inhibitors have been developed, because the sulfamate-bearing steroidal and nonsteroidal STS inhibitors may also interact with CAII [4]. Recently a novel series of 2-substituted estradiol bis-sulfamates has been developed as multitargeted antitumor agents, and the lead compound 2-methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol (**5**) is a potent irreversible STS inhibitor with 39 nM IC_{50} value, and also a highly active reversible CAII inhibitor with 379 nM IC_{50} value [3]. Although compound **5** is a dual-function STS and CAII inhibitor, its selectivity to inhibit STS over CAII is ~10-fold. This compound exhibited potent antiproferative activity with mean graph midpoint value of 87 nM in the NCI 60-cell-line panel, and GI_{50} (μM) values were 0.25 (breast MCF-7), 0.051 (lung HOP-62), 0.045 (colon HCT-116), 0.036 (CNS SF-539), <0.01 (melanoma UACC-62), <0.01 (ovarian OVCAR-3), 0.126 (renal SN12-C), 0.083 (prostate DU-145) and <0.01 (breast MDA-MB-435), respectively, [3]. Compound **5** also exhibited antiangiogenic in vitro and in vivo activity in an early-stage Lewis lung model as well; in addition, X-ray crystallography of the cocrystallization of compound **5** with CAII revealed unexpected coordination of the 17β-*O*-sulfamate of **5** to the active site zinc and a probable additional lower affinity binding site [3]. Both STS and CAII are attractive targets for molecular imaging of cancer. We are interested in the development of enzyme- and/or receptor-based cancer imaging agents for biomedical imaging technique positron emission tomography (PET), especially in dual-function imaging agents. However, the low nanomolar IC_{50} value of **5** for STS is likely on the usable range to label this compound as an imaging agent, the high nanomolar IC_{50} value of **5** for CAII is almost too

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large to be useful although the enzyme CAII is abundant, and the biological activity of **5** is not appropriate to detect/image both enzymes STS and CAII with a single agent. Therefore, we hypothesized 2-substituted estradiol bis-sulfamates labeled with a positron-emitting radionuclide such as carbon-11 or fluorine-18 may enable non-invasive monitoring of the enzyme STS and cancer response to enzyme inhibitor treatment using PET. Steroidal compound 16α-[18F]fluoro-17β-estradiol ([^{18}F]FES), as shown in Fig. 1, has been previously developed as a PET tracer to identify estrogen-receptor-positive (ER$^+$) breast tumors that are likely to respond to anti-estrogen therapy in patients [5], to delineate the ER expression in primary and metastatic breast cancer and to evaluate the therapeutic efficacy of breast cancer treated with aromatase inhibitors (AIs) [6]. In our previous works, we have synthesized carbon-11-labeled nonsteroidal sulfamate derivatives (Fig. 1) as potential PET agents for imaging of steroid biosynthetic enzymes aromatase and STS expression in breast cancer [7]. In this ongoing studies, the representative 2-substituted estradiol bis-sulfamate **5** was selected as the target compound for radiolabeling, due to its excellent biological activity and *O*-methyl position amendable to labeling with carbon-11, and we report here the first design and synthesis of 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol ([^{11}C]**5**) (Fig. 1) as a new potential imaging agent for PET to image the enzyme STS in cancers.

2. Experimental

2.1. General

All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific and used without further purification. [^{11}C]Methyl triflate ([^{11}C]CH_3OTf) was prepared according to a literature procedure [8]. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance II 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS δ 0.0), and coupling constants (J) were reported in hertz (Hz). The high resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are indicated in a volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5 × 10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20 × 20 cm^2). Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical high performance liquid chromatography (HPLC) was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:1 CH_3CN:MeOH:20 mM, pH 6.7 phosphate (buffer solution) mobile phase; 1.5 mL/min flow rate; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm, 10 × 250 mm C-18 column; 52:48 CH_3CN/H_2O mobile phase; 4.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).

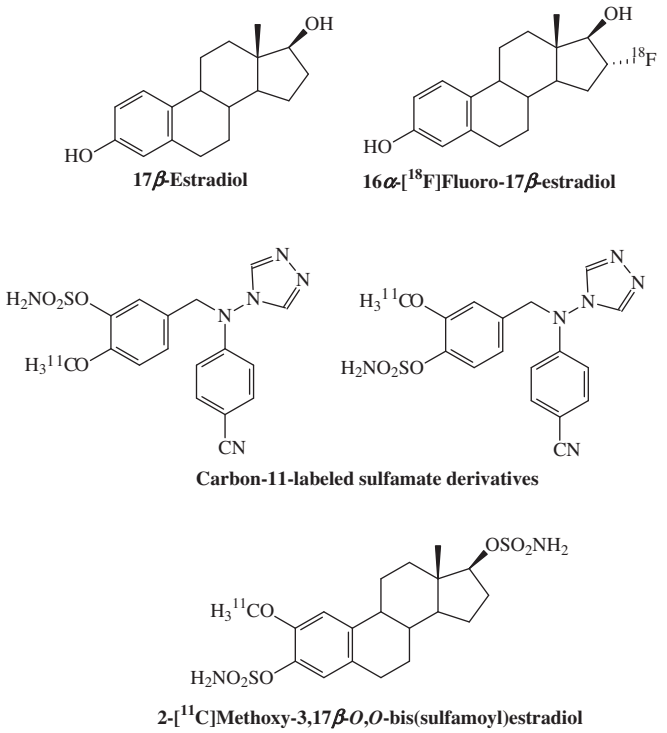


Fig. 1. Chemical structures of 17β-estradiol, 16α-[^{18}F]fluoro-17β-estradiol, carbon-11-labeled nonsteroidal sulfamate derivatives, and 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol.

2.2. 3,17β-O,O-Bis(methoxymethyl)estradiol (**1**)

Compound **1** was prepared from 17β-estradiol as a colorless oil in 94% yield. ^{1}H NMR ($CDCl_3$): δ 7.20 (d, J = 8.5 Hz, 1H), 6.82 (dd, J = 2.5, 8.5 Hz, 1H), 6.77 (d, J = 7.5 Hz, 1H), 5.14 (s, 2H), 4.67, 4.65 (ABq, J = 6.5 Hz, 2H), 3.61 (t, J = 8.5 Hz, 1H), 3.47 (s, 3H), 3.37 (s, 3H), 2.86–2.83 (m, 2H), 2.29–2.26 (m, 1H), 2.22–2.16 (m, 1H), 2.08–2.04 (m, 1H), 2.01–1.97 (m, 1H), 1.89–1.85 (m, 1H), 1.70–1.18 (m, 8H), 0.81 (s, 3H).

2.3. 2-Hydroxy-3,17β-O,O-bis(methoxymethyl)estradiol (**2**)

Compound **2** was prepared from **1** as a pale yellow oil in 84% yield. ^{1}H NMR ($CDCl_3$): δ 6.88 (s, 1H), 6.78 (s, 1H), 5.87 (br s, 1H), 5.15 (s, 2H), 4.67, 4.64 (ABq, J = 6.5 Hz, 2H), 3.61 (t, J = 8.5 Hz, 1H), 3.51 (s, 3H), 3.37 (s, 3H), 2.78–2.73 (m, 2H), 2.23–2.04 (m, 3H), 2.00–1.96 (m, 1H), 1.87–1.83 (m, 1H), 1.70–1.16 (m, 8H), 0.80 (s, 3H).

2.4. 2-Methoxy-3,17β-O,O-bis(methoxymethyl)estradiol (**3**)

Compound **3** was prepared from **2** as a white solid in 93% yield, mp 74–75 °C (lit. 73–75 °C [9]). ^{1}H NMR ($CDCl_3$): δ 6.86 (s, 1H), 6.84 (s, 1H), 5.19 (s, 2H), 4.67, 4.65 (ABq, J = 6.5 Hz, 2H), 3.85 (s, 3H), 3.62 (t, J = 8.5 Hz, 1H), 3.51 (s, 3H), 3.38 (s, 3H), 2.81–2.77 (m, 2H), 2.28–2.01 (m, 4H), 2.00–1.85 (m, 1H), 1.71–1.19 (m, 8H), 0.82 (s, 3H).

2.5. 2-Methoxyestradiol (**4**)

Compound **4** was prepared from **3** as a white solid in 83% yield, mp 186–187 °C (lit. 186–187 °C [10]). ^{1}H NMR (DMSO-d_6): δ 8.58 (s, 1H), 6.78 (s, 1H), 6.43 (s, 1H), 4.49 (d, J = 3.5 Hz, 1H), 3.71 (s, 3H), 3.54–3.50 (m, 1H), 2.65–2.59 (m, 2H), 2.27 (d, J = 11.0 Hz, 1H), 2.09–2.05 (m, 1H), 1.92–1.75 (m, 3H), 1.61–1.56 (m, 1H),1.39–1.06 (m, 7H), 0.67 (s, 3H).

*2.6. 2-Methoxy-3,17β-O,O-bis(sulfamoyl)estradiol (**5**)*

Compound **5** was prepared from **4** as a white solid in 74% yield, mp 180–182 °C (lit. 180–182 °C [3]). ^{1}H NMR (CD_3OD): δ 7.00 (s, 1H), 6.98 (s, 1H), 4.43 (t, J = 8.5 Hz, 1H), 3.82 (s, 3H), 2.79–2.77 (m, 2H), 2.38–2.35 (m, 1H), 2.29–2.24 (m, 2H), 2.08–2.05 (m, 1H), 1.91–1.87 (m, 1H), 1.84–1.74 (m, 2H), 1.54–1.41 (m, 4H), 1.36–1.24 (m, 2H), 0.86 (s, 3H). ^{13}C NMR (CD_3OD): δ 151.2, 140.6, 138.6, 130.2, 124.8, 111.5, 90.0, 56.5, 50.4, 45.7, 44.3, 39.7, 37.6, 29.6, 28.9, 28.2, 27.3, 24.0, 12.1. HRMS (ESI, *m/z*): calcd for $C_{19}H_{28}N_2O_7S_2Na$ ([M + Na]$^+$) 483.1236; found 483.1242.

*2.7. 2-Formyl-3,17β-O,O-bis(methoxymethyl)estradiol (**6**)*

Compound **6** was prepared from **5** as a white solid in 81% yield, mp 88–89 °C. ^{1}H NMR ($CDCl_3$): δ 10.43 (s, 1H), 7.77 (s, 1H), 6.91 (s, 1H), 5.26 (s, 2H), 4.67, 4.65 (ABq, J = 6.5 Hz, 2H), 3.63 (t, J = 8.5 Hz, 1H), 3.52 (s, 3H), 3.38 (s, 3H), 2.91–2.88 (m, 2H), 2.40–2.37 (m, 1H), 2.17–1.88 (m, 4H), 1.70–1.32 (m, 7H), 1.22–1.18 (m, 1H), 0.80 (s, 3H).

*2.8. 2-Formylestradiol (**7**)*

Compound **7** was prepared from **6** as a white solid in 98% yield, mp 231–233 °C (lit. 231–233 °C [11]; lit. 230–232 °C [12]). ^{1}H NMR (DMSO-d_6): δ 10.42 (s, 1H), 10.13 (s, 1H), 7.56 (s, 1H), 6.67 (s, 1H), 4.51 (d, J = 4.5 Hz, 1H), 3.54–3.50 (m, 1H), 2.83–2.78 (m, 2H), 2.29–2.26 (m, 1H), 2.11–2.06 (m, 1H), 1.92–1.85 (m, 2H), 1.80–1.77 (m, 1H), 1.60–1.55 (m, 1H), 1.42–1.33 (m, 2H), 1.31–1.07 (m, 5H), 0.66 (s, 3H).

*2.9. 2-Formyl-3,17β-O,O-bis(benzyloxy)estradiol (**8**)*

Compound **8** was prepared from **7** as a white solid in 66% yield, mp 123–125 °C (lit. 124–125 °C [12]). ^{1}H NMR ($CDCl_3$): δ 10.48 (s, 1H), 7.78 (s, 1H), 7.44–7.27 (m, 10H), 6.74 (s, 1H), 5.14 (s, 2H), 4.57 (s, 2H), 3.50 (t, J = 8.5 Hz, 1H), 2.89–2.87 (m, 2H), 2.39–2.36 (m, 1H), 2.16–2.03 (m, 4H), 1.90–1.87 (m, 1H), 1.68–1.17 (m, 7H), 0.86 (s, 3H).

*2.10. 3,17β-O,O-Bis(benzyloxy)estradiol-2-yl formate (**9**)*

To a solution of compound **8** (2.6 g, 5.41 mmol) in CH_2Cl_2 (50 mL) was added *m*-CPBA (containing 77% peracid, 1.58 g, 7.04 mmol) in portions, followed by *p*-TsOH•H_2O (25 mg) under nitrogen atmosphere. After the resulting yellow solution was stirred at room temperature (RT) for 3 h, it was diluted with water and extracted with CH_2Cl_2. The organic layers were washed with 10% aqueous Na_2SO_3, brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (8:1 hexanes/CH_3COCH_3) to afford **9** (1.91 g, 71%) as a white solid, mp 137–138 °C. ^{1}H NMR ($CDCl_3$): δ 8.26 (s, 1H), 7.37–7.27 (m, 10H), 7.01 (s, 1H), 6.74 (s, 1H), 5.05 (s, 2H), 4.57 (s, 2H), 3.50 (t, J = 8.5 Hz, 1H), 2.83–2.78 (m, 2H), 2.20–2.13 (m, 2H), 2.09–2.03 (m, 2H), 1.88–1.85 (m, 1H), 1.68–1.17 (m, 8H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.8, 147.7, 139.4, 137.1, 136.8, 135.9, 133.8, 128.7, 128.4, 128.1, 127.4, 119.7, 114.5, 88.4, 71.8, 70.9, 50.3, 44.0, 43.5, 38.3, 37.9, 29.7, 28.2, 27.2, 26.5, 23.2, 11.9. HRMS (ESI, *m/z*): calcd for $C_{33}H_{36}O_4Na$ ([M + Na]$^+$) 519.2511; found 519.2527.

*2.11. 2-Hydroxy-3,17β-O,O-bis(benzyloxy)estradiol (**10**)*

To a solution of compound **9** (1.6 g, 3.22 mmol) in MeOH (40 mL) was added 10% aqueous NaOH (8.0 mL) under nitrogen atmosphere. After the reaction mixture was heated and stirred at 60 °C for 1 h, MeOH was evaporated *in vacuo*. The residue was diluted with cold water, acidified with 1 N HCl, and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (8:1 hexanes/CH_3COCH_3) to afford **10** (1.46 g, 96%) as a white solid, mp 105–106 °C. ^{1}H NMR ($CDCl_3$): δ 7.41–7.23 (m, 10H), 6.90 (s, 1H), 6.64 (s, 1H), 5.45 (br s, 1H), 5.04(s, 2H), 4.57 (s, 2H), 3.49 (t, J = 8.5 Hz, 1H), 2.82–2.71 (m, 2H), 2.23–2.00 (m, 3H), 1.86–1.83 (m, 1H), 1.69–1.17 (m, 9 H), 0.86 (s, 3H).

*2.12. 2-Triisopropylsilyloxy-3,17β-O,O-bis(benzyloxy)estradiol (**11**)*

To a solution of compound **10** (1.0 g, 2.14 mmol) and DMAP (564 mg, 4.62 mmol) in pyridine (20 mL) was added triisopropylsilyl triflate (TIPSOTf) (4.0 mL, 14.9 mmol) dropwise at 0 °C under nitrogen atmosphere. The resulting pale yellow solution was allowed to warm up to RT. After the reaction mixture was stirred at RT overnight, the reaction was quenched with MeOH (8 mL). The mixture was diluted with Et_2O, and then washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (8:1 hexanes/Et_2O) to afford **11** (1.26 g, 95%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.43–7.24 (m, 10H), 6.80 (s, 1H), 6.59 (s, 1H), 5.00 (s, 2H), 4.58 (s, 2H), 3.49 (t, J = 8.5 Hz, 1H), 2.80–2.68 (m, 2H), 2.18–2.01 (m, 4H), 1.85–1.81 (m, 1H), 1.70–1.17 (m, 11 H), 1.34 (d, J = 7.0 Hz, 18H), 0.87 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 147.8, 143.7, 139.5, 137.7, 133.0, 129.1, 128.4, 128.4, 127.9, 127.8, 127.4, 127.4, 117.5, 114.7, 88.4, 71.8, 71.1, 50.4, 44.1, 43.6, 38.6, 38.2, 29.3, 28.2, 27.5, 26.7, 23.3, 18.2, 13.0, 12.0. HRMS (ESI, *m/z*): calcd for $C_{41}H_{56}O_3SiNa$ ([M + Na]$^+$) 647.3896; found 647.3903.

*2.13. 2-Triisopropylsilyloxyestradiol (**12a**) and 3-triisopropylsilyloxy-2-hydroxyestradiol (**12b**)*

A solution of compound **11** (1.0 g, 1.60 mmol) in EtOAc (5 mL) was hydrogenated over 20% $Pd(OH)_2$/C (360 mg) at 60 psi H_2 for 8 h. The catalyst was filtered through a layer of Celite, and then the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography (8:1–4:1, then 1:1 hexanes/Et_2O) to afford a mixture of two isomers **12a** and **12b** (357 mg, 50%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 6.86, 6.76, 6.63, 6.52 (s, 2H), 5.43, 5.41 (s, 1H), 3.72 (t, J = 8.5 Hz, 1H), 2.78–2.63 (m, 2H), 2.26–2.07 (m, 4H), 1.95–1.93 (m, 1H), 1.86–1.82 (m, 1H), 1.72–1.66 (m, 1H), 1.52–1.26 (m, 11H), 1.13–1.11 (m, 18H), 0.78 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 144.8, 144.7, 140.7, 140.6, 133.7, 131.7, 130.0, 127.9, 117.5, 114.7, 114.4, 111.7, 82.0, 82.0, 50.2, 50.1, 44.2, 44.1, 43.4, 38.9, 38.8, 36.9, 36.8, 30.8, 30.7, 29.2, 29.1, 27.6, 27.5, 26.6, 26.4, 23.2, 18.1, 18.1, 12.9, 12.2.

*2.14. 2-Triisopropylsilyloxy-3,17β-O,O-bis(sulfamoyl)estradiol (**13a**) and 3-triisopropylsilyloxy-2,17β-O,O-bis(sulfamoyl)estradiol (**13b**)*

To a cold (0 °C) solution of a mixture of compounds **12a** and **12b** (280 mg, 0.63 mmol) in dimethylacetamide (DMA) (2 mL) was added sulfamoyl chloride (435 mg, 3.78 mmol) in potions at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to warm up to RT. After the resulting yellow solution was stirred at RT overnight, it was poured into ice-water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by preparative TLC plate (4:1 $CHCl_3$/CH_3COCH_3) to afford **13a** (137 mg, 36%) and **13b** (146 mg, 38%) as white solids. **13a**, mp 79–80 °C. ^{1}H NMR ($CDCl_3$): δ 7.05 (s, 1H), 6.88 (s, 1H), 4.89 (s, 2H), 4.84 (s, 2H), 4.51 (t, J = 8.5 Hz, 1H), 2.81–2.79 (m,

2H), 2.31–2.24 (m, 1H), 2.21–2.17 (m, 2H), 2.10–2.08 (m, 1H), 1.91–1.84 (m, 2H), 1.80–1.73 (m, 1H), 1.51–1.42 (m, 4H), 1.32–1.22 (m, 5H), 1.11 (dd, J = 1.5, 7.5 Hz, 18H), 0.87 (s, 3H). ^{13}C NMR ($CDCl_3$): 145.6, 140.0, 138.4, 130.5, 124.2, 118.0, 90.7, 49.3, 44.1, 43.4, 38.1, 36.5, 28.7, 27.8, 27.0, 26.1, 23.1, 18.0, 12.8, 11.8. HRMS (ESI, *m/z*): calcd for $C_{27}H_{46}N_2O_7S_2SiNa$ ([M + Na]$^+$) 625.2413; found 625.2424. **13b**, mp 101–103 °C. ^{1}H NMR ($CDCl_3$): δ 7.19 (s, 1H), 6.63 (s, 1H), 5.23 (s, 2H), 5.18 (s, 2H), 4.46 (t, J = 8.5 Hz, 1H), 2.77–2.74 (m, 2H), 2.27–2.22 (m, 2H), 2.04–2.02 (m, 2H), 1.86–1.82 (m, 2H), 1.72–1.70 (m, 1H), 1.47–1.35 (m, 4H), 1.30–1.22 (m, 5H), 1.09 (d, J = 7.5 Hz, 18H), 0.84 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 145.7, 138.5, 136.5, 133.7, 120.8, 120.8, 90.3, 49.1, 43.6, 43.3, 38.1, 36.3, 29.2, 27.7, 26.9, 25.9, 23.1, 22.9, 17.9, 12.8, 11.8. HRMS (ESI, *m/z*): calcd for $C_{27}H_{46}N_2O_7S_2SiNa$ ([M + H]$^+$) 603.2594; found 603.2623.

2.15. 2-Hydroxy-3,17β-O,O-bis(sulfamoyl)estradiol (**14a**)

To a cold (0 °C) solution of compound **13a** (80 mg, 0.13 mmol) in THF (1.5 mL) was added n-Bu_4NF (1 M in THF, 0.4 mL, 0.4 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to warm up to RT. After the resulting yellow solution was stirred at RT overnight, it was poured into ice-water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by preparative TLC plate (4:1:0.1 $CHCl_3$/CH_3COCH_3/MeOH) to afford **14a** (47 mg, 79%) as a white solid, mp 74–75 °C. ^{1}H NMR (CD_3OD): δ 6.98 (s, 1H), 6.89 (s, 1H), 4.43 (t, J = 8.5 Hz, 1H), 2.77–2.76 (m, 2H), 2.32–2.24 (m, 2H), 2.19–2.17 (m, 1H), 2.06–2.02 (m, 1H), 1.90–1.74 (m, 3H), 1.50–1.36 (m, 4H), 1.35–1.23 (m, 2H), 0.86 (s, 3H). ^{13}C NMR (CD_3OD): δ 148.2, 140.7, 137.3, 129.3, 124.3, 115.2, 90.1, 50.4, 45.3, 44.2, 39.6, 37.5, 29.5, 28.8, 28.2, 27.2, 24.0, 12.1. HRMS (ESI, *m/z*): calcd for $C_{18}H_{26}N_2O_7S_2Na$ ([M + Na]$^+$) 469.1079; found 469.1097.

2.16. 3-Hydroxy-2,17β-O,O-bis(sulfamoyl)estradiol (**14b**)

To a cold (0 °C) solution of compound **13b** (110 mg, 0.18 mmol) in THF (2 mL) was added n-Bu_4NF (1 M in THF, 0.55 mL, 0.55 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was allowed to warm up to RT. After the resulting yellow solution was stirred for 30 min, it was poured into ice-water and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by preparative TLC plate (2:1:0.3 $CHCl_3$/CH_3COCH_3/MeOH) to afford **14b** (56 mg, 69%) as a white solid, mp 162–163 °C. ^{1}H NMR (CD_3OD): δ 7.18 (s, 1H), 6.64 (s, 1H), 4.42 (t, J = 8.0 Hz, 1H), 2.79–2.77 (m, 2H), 2.30–2.22 (m, 2H), 2.19–2.17 (m, 1H), 2.06–2.01 (m, 1H), 1.90–1.75 (m, 3H), 1.49–1.22 (m, 6H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 148.3, 137.5, 137.2, 133.0, 121.5, 118.1, 90.1, 50.4, 45.0, 44.3, 39.8, 37.5, 30.1, 28.9, 28.2, 27.2, 24.0, 12.1. HRMS (ESI, *m/z*): calcd for $C_{18}H_{26}N_2O_7S_2Na$ ([M + Na]$^+$) 469.1079; found 469.1096.

2.17. 2-[^{11}C]Methoxy-3,17β-O,O-bis(sulfamoyl)estradiol ([^{11}C]**5**)

[^{11}C]CO_2 was produced by the ^{14}N(p,α)11C nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN. Typical irradiations used for the development were 50 μA beam current and 15 min on target. The production run produced approximately 25.9 GBq of [^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor **14a** (0.3–0.5 mg) was dissolved in CH_3CN (400 μL). To this solution was added 2 N NaOH (2 μL). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method [8] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at RT, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (0.1 M, 1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2), followed by 10 mL saline, to release [^{11}C]**5**. The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **14a** = 4.85 min, t_R **5** = 7.53 min, t_R [^{11}C]**5** = 7.53 min. Retention times in the analytical HPLC system were: t_R **14a** = 3.12 min, t_R **5** = 5.34 min, t_R [^{11}C]**5** = 5.34 min. The decay corrected radiochemical yields of [^{11}C]**5** from [^{11}C]CO_2 were 40–50%.

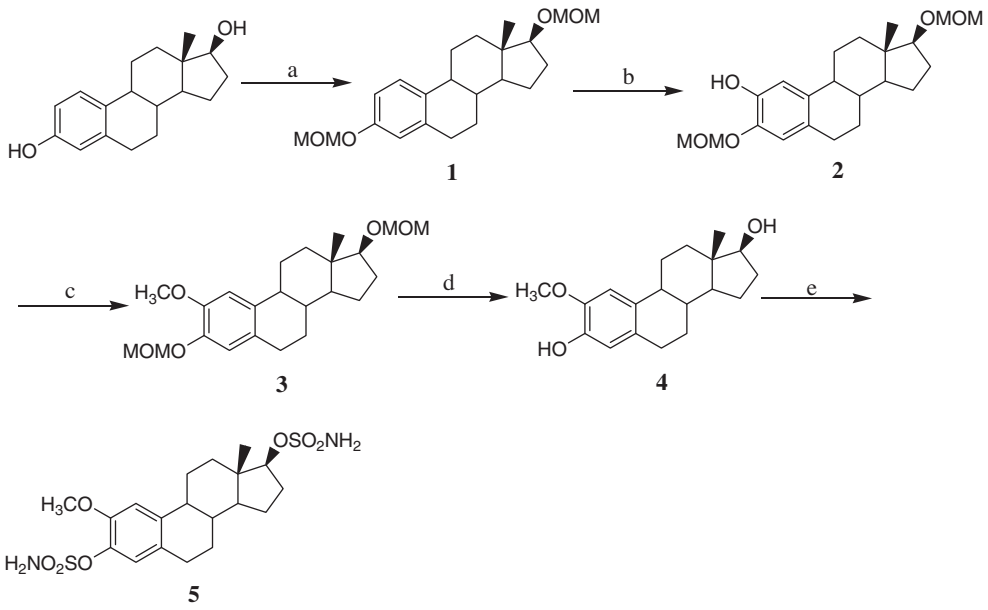
3. Results and discussion

3.1. Chemistry and lipophilicity

The reference standard **5** was synthesized from 17β-estradiol according to reported methods [3,9,13,14] in 5 steps with 40% overall chemical yield, as shown in Scheme 1.

The measured HPLC lipophilicity coefficient (octanol–water partition coefficient, Log P) is an important parameter in selecting PET radioligand candidates (small organic molecules) for further evaluations [15–18]. Since biodistributions of labeled compounds are very dependent on the lipid solubility and the more lipid soluble a compound is the longer it takes to clear from the background tissue. PET radionuclide carbon-11 does not give the long half-life to observe the process and a high Log P can give very slow clearance rates. It is a common phenomenon that an increase in lipophilicity is correlated to increasing nonspecific binding affinity of a ligand to its target protein, and when the ligand is an enzyme inhibitor, an increase in in vitro inhibitory activity is often expected when the lipophilicity of the inhibitor is increased [19]. Log P of **5** measured by analytical HPLC is 2.54, and Log P of **5** calculated from ChemDraw Ultra 9.0 (ChemOffice 2005) is 2.69. Both measured and calculated Log P values of **5** are comparable to what had reported for the STS inhibitors [19] and appropriate for **5** to be labeled as a radioligand, based on our previous experiences in radioligand development [15–18].

Direct demethylation of the standard **5** to the desmethylated precursor for radiolabeling was failed and a multiple-step synthesis of desmethylated precursor was employed, which is outlined in Scheme 2. This synthetic strategy was allowed to differentiate 2-, 3- and 17β-hydroxyl functions with different protecting groups before executing core synthetic transformations. As depicted in Scheme 2, bis-methoxymethyl (MOM) protected ether **1** was *ortho*-lithiated predominately at 2-position over 4-position with *s*-BuLi, and the resulting anion was quenched with DMF [20] to deliver the 2-formylated estradiol **6** in 81% yield. Deprotection of the bis-MOM groups of **6** with 6 N HCl in THF [20] produced 2-formylestradiol **7** in 98% yield. Estradiol **7** was protected with 3- and 17β-benzyl ether groups by treatment with BnBr in the presence of NaH and a catalytic amount of n-Bu_4NI in DMF to afford bis-benzylated compound **8** in 66% yield. For this reaction to be successful, it was essential to azeotropically dry compound **7** with toluene just prior to use, then add BnBr slowly to a stirred suspension of **7** and NaH in

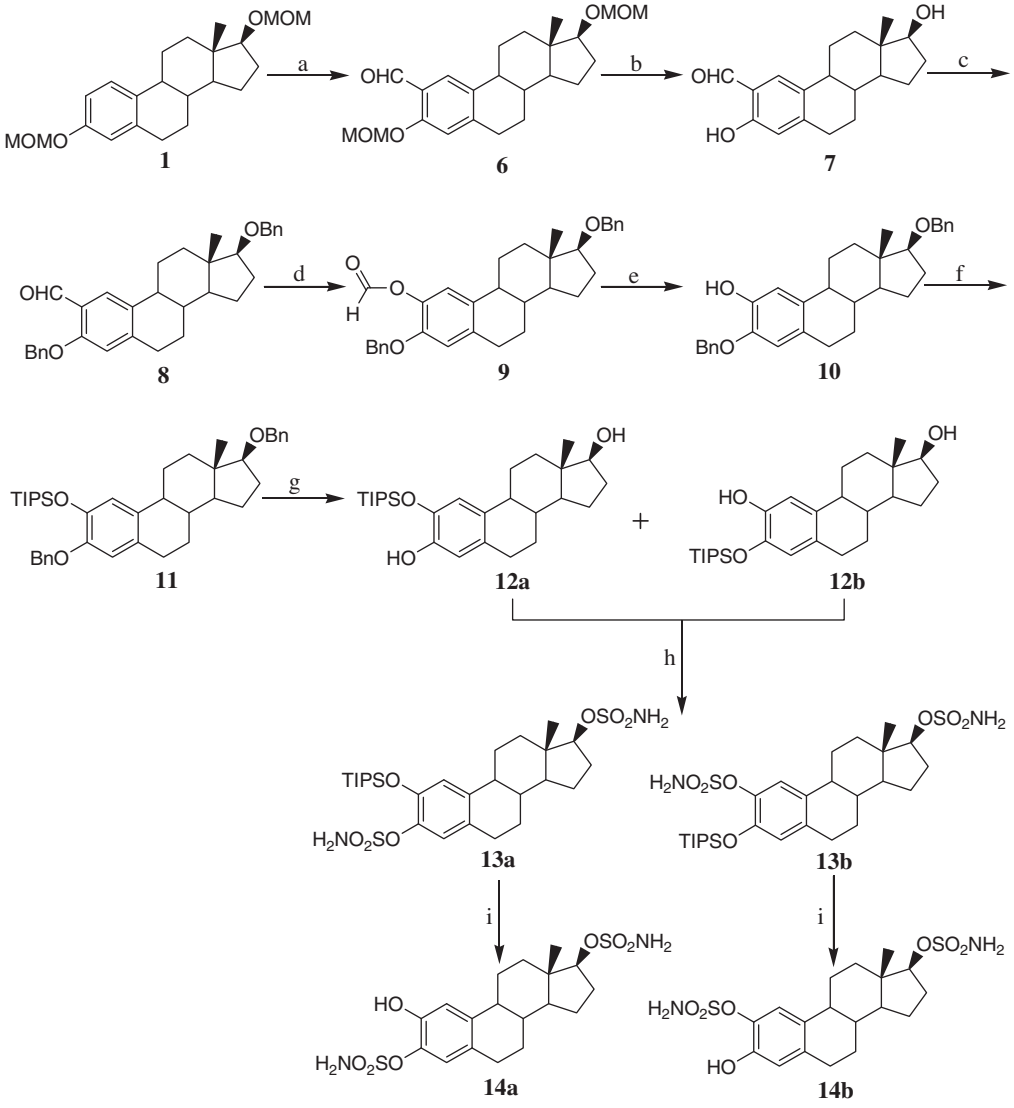


Scheme 1. Synthesis of 2-methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol (**5**). Reagents, conditions and yields: (a) *N*, *N*-diisopropylethylamine, CH_3OCH_2Cl, THF, reflux, 94%; (b) (1) *s*-BuLi, THF, −78 °C; (2) $(MeO)_3B$, −78 °C-RT; (3) $NaBO_3$, RT; 84%; (c) K_2CO_3, CH_3I, *n*-Bu_4NI, DMF, RT, 93%; (d) 6 N HCl, THF, RT, 74%; (e) sulfamoyl chloride, DMA, 0 °C-RT, 74%.

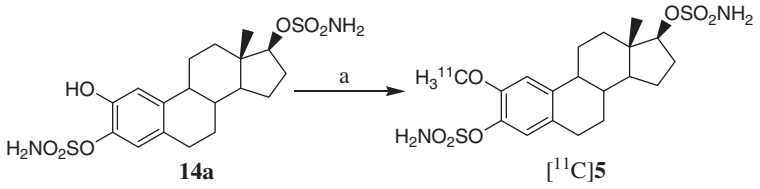
DMF at 0 °C, followed by *n*-Bu_4NI. Baeyer–Villiger oxidation of **8** with *m*-CPBA (77%) and a catalytic amount of *p*-TsOH•H_2O in CH_2Cl_2 following the procedure of Cushman et al. [12] failed to directly give phenol **10**, but gave formyl ester **9** instead in 71% yield. We noticed that Cushman et al. probably did not attempt to isolate the intermediate formate ester **9** and hydrolyzed it in situ to the phenol **10**. At the beginning, we thought the reason could be they used 80–90% *m*-CPBA in the reaction, and commercially available *m*-CPBA we purchased was only 77%. Then we purified the purchased *m*-CPBA to increase the concentration of peracid to 80–90% and exactly repeated the reported procedure [12] again, the product was still formate ester **9**, and no phenol **10** was identified. It is likely the basic condition is necessary for the hydrolysis of **9** to **10**. The reported procedure for Baeyer–Villiger oxidation was conducted under acidic condition (*p*-TsOH). It might not be possible to hydrolyze **9** in situ to the phenol **10**. Compound **9** was then hydrolyzed by treatment with 10% NaOH in MeOH to afford phenol **10** in 96% yield. Access to precursor compound **14a** required a sequence of silyl protection of 2-hydroxy group and debenzylation of 3- and 17β-benzyl ether groups of compound **10**. Considering that triisopropylsilyl (TIPS) group is less prone to migrate to proximate hydroxyl group than the *tert*-butyldimethylsilyl (TBDMS) group [7] for the following hydrogenolysis, attempt to protect 2-hydroxyl group of compound **10** with triisopropylsilyl chloride (TIPSCl) as silylating agent proved unsuccessful. TIPS protected ether **11** was achieved with another silylating agent TIPSOTf in the presence of DMAP in pyridine in 95% yield. Debenzylation of the bis-benzyl groups by hydrogenolysis with 10% Pd/C as catalyst only removed 3-benzyl protecting group. Cleavage of the bis-benzyl groups was accomplished over another catalyst 20% $Pd(OH)_2$/C in EtOAc to yield a mixture of two regioisomers **12a** and **12b** in 50% yield, which were difficult to separate by column chromatography. The ratio of the resulting regioisomers **12a** and **12b** was determined by ^1H NMR as 1:1, this indicated that **12b** is the product of 1,2-*O*,*O*-TIPS migration of **12a** [7]. Without isolation, the phenols mixture was sulfamoylated with sulfamoyl chloride in DMA to give sulfamate regioisomers **13a** and **13b**, which were readily separated by column chromatography in 36% and 38% yield, respectively. Desilylation of **13a** and **13b** with tetrabutylammonium fluoride (TBAF, *n*-Bu_4NF) in THF gave **14a** and **14b** in 79% and 69% yield, respectively. The overall chemical yield for the precursor **14a** starting from 17β-estradiol in 10 steps was 5%.

3.2. Radiochemistry

Synthesis of the target tracer carbon-11-labeled bis-sulfamate [^{11}C]**5** is indicated in Scheme 3. The precursor **14a** was labeled by [^{11}C]CH_3OTf [8,21] through *O*-[^{11}C]methylation [22,23] at 80 °C under basic condition (2 N NaOH) and isolated by a semi-preparative HPLC with a C-18 column and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge (a second purification or isolation process) [24–26] to produce the corresponding pure radiolabeled compound [^{11}C]**5** in 40–50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. [^{11}C]CH_3OTf is a proven methylation reagent with greater reactivity than commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I) [27], and thus, the radiochemical yield of [^{11}C]**5** was relatively high. Addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [^{11}C]**5** from its phenol hydroxyl precursor **14a** [24–26,28]. The radiosynthesis was performed in a home-built automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific radioactivity during synthesis [29,30]. This ^{11}C-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, ^{11}C-tracer specific activity (GBq/μmol at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system. The overall synthesis, purification and reformulation time was 30–40 min from EOB. The specific radioactivity was in a range of 370–740 GBq/μmol at EOB. Chemical purity and radiochemical purity were determined by analytical HPLC [31]. The chemical purity of the precursor **14a** and reference standard **5** was >96%. The radiochemical purity of the target tracer [^{11}C]**5** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]**5** was >93% determined by reverse-phase HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge was used to significantly improve



Scheme 2. Synthesis of 2-hydroxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol (**14a**). Reagents, conditions and yields: (a) (1) *s*-BuLi, THF, -78 °C; (2) DMF, -78 °C-RT; 81%; (b) 6 N HCl, THF, RT, 98%; (c) NaH, BnBr, *n*-Bu_4NI, DMF, 0 °C-RT, 66%; (d) *m*-CPBA, *p*-TsOH•H_2O, CH_2Cl_2, RT, 71%; (e) 10% NaOH, MeOH, 60 °C, 96%; (f) DMAP, TIPSOTf, pyridine, 0 °C-RT, 95%; (g) H_2, 20% $Pd(OH)_2$/C, EtOAc, 60 psi, RT, 50% (1:1 **12a**/**12b**); (h) sulfamoyl chloride, DMA, 0 °C-RT, 36% (**13a**), 38% (**13b**); (i) *n*-Bu_4NF, THF, RT, 79% (**14a**), 69% (**14b**).



Scheme 3. Synthesis of 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol ([^{11}C]**5**). Reagents, conditions and yields: (a) [^{11}C]CH_3OTf, CH_3CN, 2 N NaOH, 80 °C, 3 min, 40–50%.

the chemical purity of the tracer solution. Initial HPLC purification was employed to separate the labeled product from its un-reacted excess precursor and other labeled by-products. The second SPE purification with Sep-Pak [24–26,31] was employed, instead of rotary evaporator, to remove potential impurities from the HPLC co-elution with the precursor and from the residual solvents including HPLC mobile phase solvents and module set-up cleaning solvents. Rotary evaporation was unable to perform in this regard. Moreover, it could result in the decomposition of the labeled product such as desmethylation during the heating. The chemical purity of the [^{11}C]5 tracer solution with Sep-Pak purification was increased higher 10–20% than that without Sep-Pak purification [24–26].

4. Conclusion

In summary, an efficient and convenient synthesis of a new carbon-11-labeled 2-substituted estradiol bis-sulfamate, 2-[^{11}C]methoxy-3,17β-*O*,*O*-bis(sulfamoyl)estradiol, has been developed. The

reference standard, the precursor for labeling, and new 2-substituted estradiol derivatives have been synthesized. The target radiotracer was prepared by the *O*-[^{11}C]methylation of its corresponding phenolic precursor using a reactive [^{11}C]methylating agent, [^{11}C]CH_3OTf, and isolated by a HPLC combined with SPE procedure in high radiochemical yields, short overall synthesis time, and great specific radioactivity. These chemistry results combined with the reported in vitro and in vivo biological data [3] encourage further in vivo biological evaluation of new carbon-11-labeled 2-substituted estradiol bis-sulfamate as a candidate PET agent for imaging of STS in cancers.

Acknowledgments

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