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Design and synthesis of carbon-11-labeled dual aromatase–steroid sulfatase inhibitors as new potential PET agents for imaging of aromatase and steroid sulfatase expression in breast cancer

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ABSTRACT

Aromatase and steroid sulfatase (STS) are particularly attractive targets in the treatment of estrogen-receptor-positive breast cancer and the development of enzyme-based cancer imaging agents for the biomedical imaging technique positron emission tomography (PET). New carbon-11-labeled sulfamate derivatives were first designed and synthesized as potential PET dual aromatase–steroid sulfatase inhibitor (DASSI) radiotracers for imaging of aromatase and STS expression in breast cancer. The target tracers 5-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8a**) and 4-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8b**) were prepared from their corresponding precursors 5-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-hydroxyphenyl sulfamate (**16**) and 4-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-hydroxyphenyl sulfamate (**21**) with [^{11}C]CH_3OTf under basic conditions through the *O*-[^{11}C]methylation and isolated by the reversed-phase high pressure liquid chromatography (HPLC) method in 30–45% radiochemical yields based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The specific activity at end of synthesis (EOS) was 111–185 GBq/μmol.

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1. Introduction

Breast cancer is the most common cancer diagnosed in women and is the second leading cause of death in women after lung cancer in the United States [1]. The exact cause of the breast cancer remains unknown. Most breast cancer patients will survive after conventional treatment modalities such as surgery, radiation therapy, hormone therapy, chemotherapy and/or a combination thereof if the breast cancer can be detected at the early stage [2]. However, these treatments still fail to cure a significant number of patients. Rapid and accurate early detection is highly desirable so that various therapeutic regiments can be given before the primary tumors become widely spread [3]. In the diagnosis and treatment of breast cancer, the biomedical imaging technique positron emission tomography (PET) has become a clinically valuable and accepted new medical imaging tool [4]. PET coupled with radiopharmaceutical 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is widely used in clinical settings to diagnose breast cancer [5]. [^{18}F]FDG is the only PET breast cancer imaging agent used clinically at this point in time. However, [^{18}F]FDG is not in all cases satisfactory. There are some well-known limitations to its use such as the inability of [^{18}F]FDG-PET to visualize very small breast tumors and the low cellular uptake rate of [^{18}F]FDG in breast cancer [6]. In addition, only a limited number of PET studies using other radiotracers have been conducted to image breast cancer and monitor its response to treatment, due to the limited accessibility of radiotracers. These limitations have motivated the development of new breast cancer PET imaging agents. Radiotracer development is a key area for advancement of research and clinical applications of PET in cancer detection and treatment [7].

The improved therapies with fewer side effects and early detection with advanced imaging technology may help to decrease the overall death rate of breast cancer. Novel strategies are eagerly needed. To this end, aromatase inhibitor (AI) therapy has received wide attention in treating hormone-dependent breast cancer (HDBC) [8], since AIs are equivalent or superior to tamoxifen, a well-known selective estrogen receptor modulator (SERM), and currently in advanced clinical trials or in the market for treatment of HDBC, as first-line therapy for metastatic breast cancer and as neoadjuvant treatment for primary breast cancer. Moreover, AIs can be administered instead of tamoxifen as a single agent for 5 years or sequentially with tamoxifen for 5 or 10 years as shown in

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the randomized studies [9]. The clinical efficacy of AIs in the treatment of HDBC has clearly been demonstrated. Furthermore, there are evidences to suggest that the deprivation of estrogen levels in patients treated with AIs can be augmented if the enzyme steroid sulfatase (STS) is inhibited simultaneously, since the enzyme aromatase converts androgen to estrogen, the last and rate-limiting step in the estrogen biosynthesis; and the enzyme STS catalyzes the hydrolysis of steroid sulfates to estrones, the main source of estrogens in tumors [10]. Therefore, STS inhibitors coupled with AIs may enhance the response of HDBC to the hormonal therapy. The enzymes aromatase and STS are particularly attractive targets in the development of both therapeutic agents and diagnostic agents for HDBC. A novel series of sulfamate derivatives have been recently developed as dual aromatase–steroid sulfatase inhibitors (DASSIs) [11]. Sulfamate derivatives labeled with a positron-emitting radionuclide such as carbon-11 or fluorine-18 may enable non-invasive monitoring of steroid biosynthetic enzymes aromatase and STS and breast cancer response to DASSI treatment using PET. Two selected title compounds, 5-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-methoxyphenyl sulfamate (**8a**) and 4-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-methoxyphenyl sulfamate (**8b**), exhibited *in vitro* inhibition of aromatase and STS activity assessed using intact monolayers of JEG-3 cells with IC_{50} (nM) 12 ± 1.9 and >10,000, and 42 ± 1 and 380 ± 31, respectively [11]. Also, they possess *O*-methyl position amenable to labeling with carbon-11. These same properties are often beneficial in a diagnostic radiotracer.

We are interested in the development of enzyme- and/or receptor-based PET breast cancer imaging agents. Steroidal compound 16α-[^{18}F]fluoro-17β-estradiol ([^{18}F]FES), as shown in Fig. 1, has been developed as a PET tracer to identify estrogen-receptor-positive (ER^+) breast tumors that are likely to respond to anti-estrogen therapy in patients [12], to delineate the ER expression in primary and metastatic breast cancer and to evaluate the therapeutic efficacy of breast cancer treated with AIs [13]. However, [^{18}F]FES requires complex radiosynthesis. Compared to the fluorine-18 tracers (half-life 110 min), the carbon-11 tracers (half-life 20 min) have some advantages in back-to-back same-day PET studies, such as avoiding movement of the subject from the PET scanner and performing another study within 2–3 h to explore

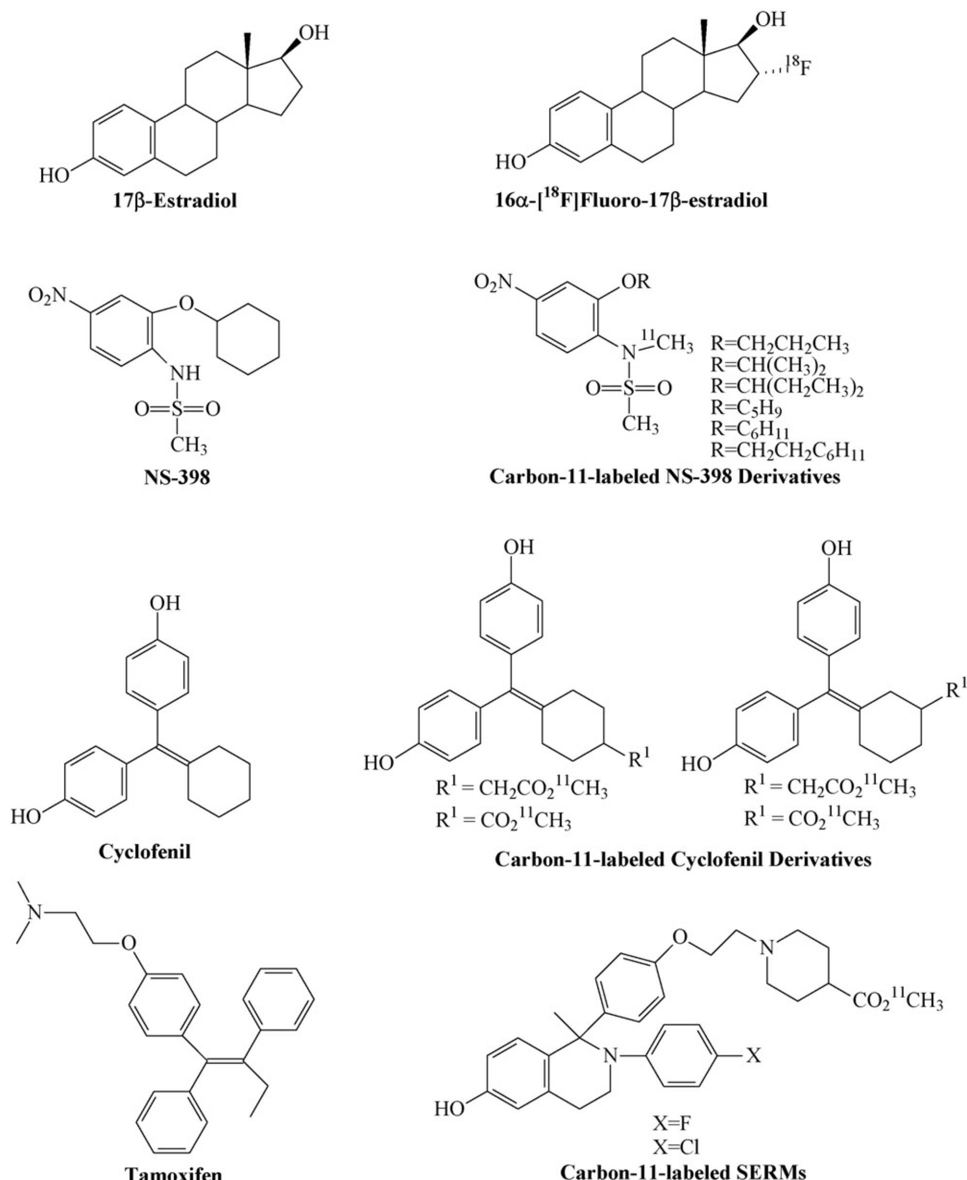


Fig. 1. Enzyme- and receptor-based PET breast cancer imaging agents.

drug effects at the first study. These advantages become very valuable in studying pharmacological or behavioral changes [14]. We focus on the development of carbon-11-labeled non-steroidal analogs that are inhibitors of steroid biosynthetic enzymes or ligands for steroid hormone receptors. In our previous works, we have developed carbon-11-labeled AIs (NS-398 derivatives) for imaging of aromatase enzyme [15], carbon-11-labeled cyclofenil derivatives as PET non-steroidal estrogen radioligands for imaging of ER [16], and carbon-11-labeled tetrahydroisoquinoline derivatives as SERM radioligands for imaging of ER expression [17] in breast cancer (Fig. 1). This ongoing study was to develop new DASSI radiotracers. To further develop therapeutic agent for diagnostic use, we have first designed and synthesized carbon-11-labeled sulfamate derivatives, 5-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8a**) and 4-(((4-cyanophenyl)(4*H*-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8b**), as new potential PET agents for imaging of aromatase and STS expression in breast cancer.

2. Experimental

2.1. General

All commercial reagents and solvents from Aldrich and Sigma were used without further purification. [^{11}C]methyl triflate ([^{11}C]CH_3OTf) was prepared according to a literature procedure [18]. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance II 500 MHz NMR spectrometers 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). High resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are indicated as volume:volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates ($5 \times 10\,cm^2$). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20 cm × 20 cm). 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 pressure liquid chromatography (HPLC) was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 mm × 250 mm; 3:1:1 CH_3CN:MeOH:20 mM, pH 6.7 phosphate (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10 mm × 250 mm C-18 column; 3:1:1 CH_3CN:MeOH:20 mM, pH 6.7 phosphate (buffer solution) mobile phase; 5.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

2.2. Synthesis of sulfamate derivatives

2.2.1. 3-Benzyloxy-4-methoxybenzaldehyde (2a)

To a stirred suspension of NaH (60% dispersion in mineral oil, 4.5 g, 112.5 mmol) in DMF (200 mL) at 0 °C under N_2 was added a solution of 3-hydroxy-4-methoxybenzaldehyde (**1a**) (15.0 g, 98.6 mmol) in DMF (100 mL), followed by benzyl bromide (14.1 mL, 118.0 mmol). After stirring at room temperature for 4 h, the reaction mixture was quenched with cold water, neutralized with 1N HCl and extracted with Et_2O. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:3 EtOAc/hexanes) to give **2a** (19.8 g, 83%) as a pale yellow solid, m.p. 63–65 °C (lit. 61–63 °C [19]). ^{1}H NMR ($CDCl_3$) δ: 9.82 (s, 1H), 7.43–7.29 (m, 7H), 7.00 (d, J = 8.7 Hz, 1H), 5.20 (s, 2H), 3.97 (s, 3H).

2.2.2. 4-Benzyloxy-3-methoxybenzaldehyde (2b)

The title compound **2b** was obtained from vanillin (**1b**) (15.0 g, 98.6 mmol) following the procedure as described in the preparation of **2a** as a pale yellow solid (18.3 g, 77%), m.p. 60–61 °C (lit. 62–64 °C [20]). ^{1}H NMR ($CDCl_3$) δ: 9.82 (s, 1H), 7.43–7.30 (m, 7H), 6.97 (d, J = 8.0 Hz, 1H), 5.22 (s, 2H), 3.92 (s, 3H).

2.2.3. (3-(Benzyloxy)-4-methoxyphenyl)methanol (3a)

To a stirred solution of **2a** (10.0 g, 41.3 mmol) in MeOH (80 mL) was added $NaBH_4$ (2.0 g, 52.9 mmol) portionwise at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was concentrated in vacuo. To the residue was added cooled water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give **3a** (9.45 g, 93%) as a white solid, which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.45 (d, J = 7.0 Hz, 2H), 7.38–7.30 (m, 2H), 7.31 (d, J = 7.0 Hz, 1H), 6.96 (d, J = 2.0 Hz, 1H), 6.92–6.86 (m, 2H), 5.16 (s, 2H), 4.57 (s, 2H), 3.88 (s, 3H).

2.2.4. (4-(Benzyloxy)-3-methoxyphenyl)methanol (3b)

The title compound **3b** was obtained from **2b** (10.0 g, 41.3 mmol) following the procedure as described in the preparation of **3a** as a white solid (9.9 g, 97%), which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.43 (d, J = 7.0 Hz, 2H), 7.38–7.30 (m, 3H), 6.95 (d, J = 2.0 Hz, 1H), 6.92–6.86 (m, 2H), 5.16 (s, 2H), 4.61 (s, 2H), 3.89 (s, 3H).

2.2.5. 3-(Benzyloxy)-4-methoxybenzyl bromide (4a)

To a stirred suspension of **3a** (5.0 g, 20.5 mmol) in Et_2O (50 mL) was added a solution of PBr_3 (2.5 mL, 26.5 mmol) in Et_2O (15 mL) slowly. After stirring at room temperature for 2 h, the reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to afford **4a** (5.85 g, 93%) as a pale yellow solid, which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.45 (d, J = 7.5 Hz, 2H), 7.39–7.29 (m, 3H), 6.97–6.95 (m, 2H), 6.83 (d, J = 8.0 Hz, 1H), 5.14 (s, 2H), 4.45 (s, 2H), 3.88 (s, 3H).

2.2.6. 4-(Benzyloxy)-3-methoxybenzyl bromide (4b)

The title compound **4b** was obtained from **3b** (5.0 g, 20.5 mmol) following the procedure as described in the preparation of **4a** as a pale yellow solid (5.93 g, 94%), which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.42 (d, J = 7.5 Hz, 2H), 7.38–7.29 (m, 3H), 6.93 (d, J = 1.5 Hz, 1H), 6.89–6.87 (m, 1H), 6.81 (d, J = 8.5 Hz, 1H), 5.15 (s, 2H), 4.48 (s, 2H), 3.90 (s, 3H).

2.2.7. 4-((4-Cyanophenyl)amino)-4H-1,2,4-triazole (5)

To a stirred suspension of potassium *tert*-butoxide (22.4 g, 200 mmol) in DMSO (100 mL) was added 4-amino-4*H*-1,2,4-triazole (16.8 g, 200 mmol) portionwise at 0 °C. After stirring at room temperature for 0.5 h, a solution of 4-fluorobenzonitrile (12.2 g, 100 mmol) in DMSO (30 mL) was added slowly below 30 °C. The mixture was stirred for additional 0.5 h at room temperature, then poured into water and neutralized with 1N HCl. The precipitate was filtered and recrystallized from water to afford **5** (7.80 g,

42%) as a white solid, m.p. 202–204 °C (lit. 206–208 °C [21]). ^{1}H NMR (DMSO-d_6) δ: 10.22 (s, 1H), 8.84 (s, 2H), 7.69 (d, J = 9.0 Hz, 2H), 6.56 (d, J = 9.0 Hz, 2H).

*2.2.8. 4-((3-(Benzyloxy)-4-methoxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**6a**)*

To a stirred suspension of NaH (60% dispersion in mineral oil, 880 mg, 22.0 mmol) in DMF (25 mL) was added **5** (4.0 g, 21.6 mmol) portionwise at 0 °C. After stirring at room temperature for 0.5 h, a solution of **4a** (5.04 g, 16.5 mmol) in DMF (10 mL) was added and the mixture was heated at 90 °C overnight. The reaction mixture was cooled, poured into water, and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was recrystallized from EtOH to afford **6a** (5.24 g, 78%) as a white solid, m.p. 148–150 °C (lit. 147–149 °C [11]). ^{1}H NMR (DMSO-d_6) δ: 8.67 (s, 2H), 7.78 (d, J = 9.0 Hz, 2H), 7.42–7.32 (m, 5H), 7.00 (d, J = 2.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.78–6.75 (m, 3H), 5.04 (s, 2H), 4.94 (s, 2H), 3.73 (s, 3H).

*2.2.9. 4-((4-(Benzyloxy)-3-methoxybenzyl)(4H-1,2,4-triazole-4-yl)amino)benzonitrile (**6b**)*

The title compound **6b** was obtained from **5** (4.0 g, 21.6 mmol) and **4b** (5.04 g, 16.5 mmol) following the procedure as described in the preparation of **6a** as a off-white solid (2.72 g, 40%) after recrystallization from *i*-PrOH, m.p. 210–212 °C (lit. 210–214 °C [11]). ^{1}H NMR (DMSO-d_6) δ: 8.77 (s, 2H), 7.76 (d, J = 9.0 Hz, 2H), 7.43–7.31 (m, 5H), 6.93 (d, J = 8.0 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 9.0 Hz, 2H), 6.44 (dd, J = 8.0, 2.0 Hz, 1H), 5.02 (s, 2H), 4.96 (s, 2H), 3.73 (s, 3H).

*2.2.10. 4-((3-Hydroxy-4-methoxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**7a**)*

To a stirred solution of **6a** (4.0 g, 9.7 mmol) in THF (200 mL) and MeOH (100 mL) was added 10% Pd/C (400 mg). The suspension was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight. The catalyst was removed by filtration through Celite. The solvent was evaporated in vacuo, and the crude product was recrystallized from EtOH to give **7a** (2.20 g, 71%) as a white solid, m.p. 224 °C (dec) (lit. > 230 °C (dec) [11]). ^{1}H NMR (DMSO-d_6) δ: 9.01 (s, 1H), 8.71 (s, 2H), 7.75 (d, J = 9.0 Hz, 2H), 6.81 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 9.5 Hz, 2H), 6.70 (d, J = 2.0 Hz, 1H), 6.64 (dd, J = 8.0, 2.0 Hz, 1H), 4.89 (s, 2H), 3.72 (s, 3H).

*2.2.11. 4-((4-Hydroxy-3-methoxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**7b**)*

The title compound **7b** was prepared by the hydrogenation of **6b** (2.6 g, 6.3 mmol) with 10% Pd/C (260 mg) in THF (100 mL) and MeOH (50 mL) following the procedure as described in the preparation of **7a** as a white solid (2.03 g, 72%) after recrystallization from *i*-PrOH, m.p. 215 °C (dec) (lit. > 220 °C (dec) [11]). ^{1}H NMR (DMSO-d_6) δ: 9.02 (br s, 1H), 8.72 (s, 2H), 7.76 (d, J = 9.0 Hz, 2H), 6.81–6.78 (m, 3H), 6.65 (d, J = 8.0 Hz, 1H), 6.44 (dd, J = 8.0, 2.0 Hz, 1H), 4.90 (s, 2H), 3.71 (s, 3H).

*2.2.12. 5-(((4-Cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-methoxyphenyl sulfamate (**8a**)*

To a stirred solution of **7a** (1.55 g, 4.83 mmol) in dimethylamine (DMA) (15 mL) was added sulfamoyl chloride (2.0 g, 17.3 mmol) at 0 °C under nitrogen atmosphere. After stirring at room temperature overnight, the mixture was poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:7 MeOH/$CHCl_3$) to give **8a** (1.43 g, 74%) as a white solid, m.p. 165 °C (dec) (lit. 169–171 °C (dec) [11]). ^{1}H NMR (DMSO-d_6) δ: 8.74 (s, 2H), 7.93 (s, 2H), 7.76 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 2.0 Hz, 1H), 7.13 (dd, J = 8.0, 2.0 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 8.5 Hz, 2H), 5.00 (s, 2H), 3.77 (s, 3H).

*2.2.13. 4-(((4-Cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-methoxyphenyl sulfamate (**8b**)*

The title compound **8b** was obtained from **7b** (420 mg, 1.25 mmol) and sulfamoyl chloride (504 mg, 4.36 mmol) in DMA (4 mL) following the procedure as described in the preparation of **8a** as a white solid (430 mg, 83%), m.p. 150 °C (dec) (lit. > 150 °C (dec) [11]). ^{1}H NMR (DMSO-d_6) δ: 8.91 (s, 2H), 7.95 (s, 2H), 7.78 (d, J = 9.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 1H), 7.09 (d, J = 1.5 Hz, 1H), 6.91 (dd, J = 8.0, 2.0 Hz, 1H), 6.78 (d, J = 8.0 Hz, 2H), 5.07 (s, 2H), 3.78 (s, 3H).

*2.2.14. 4-(Benzyloxy)-3-hydroxybenzaldehyde (**9**)*

To a stirred solution of 3,4-dihydroxybenzaldehyde (15.0 g, 109 mmol) in DMF (250 mL) was added $NaHCO_3$ (13.7 g, 163 mmol), NaI (4.9 g, 32.7 mmol) and benzyl chloride (25.1 mL, 218 mmol). After stirring at 45 °C overnight, the reaction mixture was quenched with 1N HCl (1 L) and extracted with EtOAc. The organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:4 EtOAc/hexanes) to give **9** (17.2 g, 70%) as a white solid, m.p. 119–121 °C (lit. 118–120 °C [22]). ^{1}H NMR ($CDCl_3$) δ: 9.85 (s, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.43–7.38 (m, 6H), 7.04 (d, J = 8.0 Hz, 1H), 5.21 (s, 2H).

*2.2.15. 4-(Benzyloxy)-3-(tert-butyldimethylsilyloxy)benzaldehyde (**10**)*

To a stirred solution of **9** (14.0 g, 61.4 mmol) and 4-(dimethylamino)pyridine (DMAP) (2.25 g, 18.4 mmol) in CH_2Cl_2 (200 mL) was added triethylamine (60.0 mL, 430 mmol) at 0 °C under nitrogen atmosphere, and followed by *tert*-butyldimethylsilyl chloride (27.7 g, 184.2 mmol). After stirring at 0 °C for 1 h and at room temperature for 3 h, the reaction mixture was washed with saturated NH_4Cl. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:9 hexanes/CH_2Cl_2) to give **10** (20.3 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 9.84 (s, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.45–7.33 (m, 6H), 7.02 (d, J = 8.0 Hz, 1H), 5.13 (s, 2H), 0.96 (s, 9H), 0.12 (s, 6H). HRMS (CI, *m*/*z*): calculated for $C_{20}H_{27}O_3Si$ ($[M+H]^+$) 343.1724; found 343.1717.

*2.2.16. (4-(Benzyloxy)-3-(tert-butyldimethylsilyloxy)phenyl)methanol (**11**)*

To a stirred solution of **10** (20.0 g, 58.4 mmol) in MeOH (100 mL) was added $NaBH_4$ (2.87 g, 75.9 mmol) portionwise at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was concentrated in vacuo. To the residue was added cooled water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give **11** (17.1 g, 85%) as a pale yellow oil, which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.43 (d, J = 7.0 Hz, 2H), 7.38–7.30 (m, 3H), 6.89 (s, 1H), 6.87 (s, 2H), 5.04 (s, 2H), 4.56 (s, 2H), 0.96 (s, 9H), 0.11 (s, 6H). HRMS (CI, *m*/*z*): calculated for $C_{20}H_{29}O_3Si$ ($[M+H]^+$) 345.1880; found 345.1886.

*2.2.17. 4-(Benzyloxy)-3-(tert-butyldimethylsilyloxy)benzyl bromide (**12**)*

To a stirred solution of **11** (11.8 g, 34.5 mmol) in Et_2O (150 mL) was added a solution of PBr_3 (3.4 mL, 36.2 mmol) in Et_2O (30 mL) slowly. After stirring at room temperature for 4 h, the reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to

afford **12** (10.1 g, 72%) as a pale yellow oil, which was used for next step without further purification. ^{1}H NMR ($CDCl_3$) δ: 7.45 (d, J = 7.0 Hz, 2H), 7.40 (t, J = 7.0 Hz, 2H), 7.35 (d, J = 7.0 Hz, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.0 Hz, 1H), 5.06 (s, 2H), 4.46 (s, 2H), 1.00 (s, 9H), 0.15 (s, 6H). HRMS (CI, m/z): calculated for $C_{20}H_{28}O_2BrSi$ ([M]$^+$) 407.1036; found 407.1029.

*2.2.18. 4-((4-(Benzyloxy)-3-(tert-butyldimethylsilyloxy)benzyl)(4H-1,2,4-triazole-4-yl)amino)benzonitrile (**13**)*

A mixture of **5** (3.88 g, 21.0 mmol), **12** (8.50 g, 21.0 mmol) and K_2CO_3 (5.79 g, 41.9 mmol) in CH_3CN (150 mL) was stirred at room temperature overnight. The reaction mixture was quenched with water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:30 MeOH/CH_2Cl_2) to give **13** (4.97 g, 46%) as a yellow solid, m.p. 137–139 °C. ^{1}H NMR ($CDCl_3$) δ: 8.09 (s, 2H), 7.58 (d, J = 9.0 Hz, 2H), 7.41–7.31 (m, 5H), 6.83 (d, J = 8.0 Hz, 1H), 6.71–6.66 (m, 4H), 5.01 (s, 2H), 4.77 (s, 2H), 0.91 (s, 9H), 0.03 (s, 6H). HRMS (TOF ES+, m/z): calculated for $C_{29}H_{34}N_5O_2Si$ ([M+H]$^+$) 512.2482; found 512.2502.

*2.2.19. 4-((4-(Benzyloxy)-3-hydroxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**14**)*

To a stirred solution of **13** (1.50 g, 2.94 mmol) in THF (20 mL) was added 1.0 M solution of tetra-n-butylammonium fluoride (TBAF) in THF (8.81 mL, 8.81 mmol) slowly at 0 °C under nitrogen atmosphere. After stirring at room temperature for 5 h, the reaction mixture was poured into ice-water. The precipitate was filtered and the solid was washed with cold water and Et_2O to afford **14** (1.06 g, 91%) as a pink solid, m.p. 209–211 °C. ^{1}H NMR (DMSO-d_6) δ: 9.13 (s, 1H), 8.72 (s, 2H), 7.75 (d, J = 9.0 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.32–7.30 (m, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.76–6.74 (m, 3H), 6.63 (dd, J = 8.5, 2.0 Hz, 1H), 5.05 (s, 2H), 4.89 (s, 2H). HRMS (TOF ES+, m/z): calculated for $C_{23}H_{20}N_5O_2$ ([M+H]$^+$) 398.1617; found 398.633.

*2.2.20. 2-(Benzyloxy)-5-(((4-cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (**15**)*

To a stirred solution of **14** (600 mg, 1.51 mmol) in DMA (5 mL) was added sulfamoyl chloride (520 mg, 4.53 mmol) at 0 °C under nitrogen atmosphere. After stirring at room temperature overnight, the mixture was poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:30 MeOH/$CHCl_3$) to give **15** (596 mg, 84%) as a white solid, m.p. 182–184 °C. ^{1}H NMR (DMSO-d_6) δ: 8.70 (s, 2H), 8.01 (s, 2H), 7.76 (d, J = 9.0 Hz, 2H), 7.47 (d, J = 7.0 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.33–7.30 (m, 2H), 7.12–7.08 (m, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.15 (s, 2H), 4.99 (s, 2H). HRMS (TOF ES+, m/z): calculated for $C_{23}H_{21}N_6O_4S$ ([M+H]$^+$) 477.2345; found 477.1366.

*2.2.21. 5-(((4-Cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-hydroxyphenyl sulfamate (**16**)*

To a stirred solution of **15** (350 mg, 0.74 mmol) in EtOH (5 mL) and THF (5 mL) was added 10% Pd/C (56 mg). The suspension was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight. The catalyst was removed by filtration through Celite. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:7 MeOH/CH_2Cl_2) to give **16** (47 mg, 17%) as a white solid, m.p. 149 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 9.85 (s, 1H), 8.72 (s, 2H), 7.84 (s, 2H), 7.76 (d, J = 9.0 Hz, 2H), 7.19 (d, J = 2.0 Hz, 1H), 6.97 (dd, J = 8.5, 2.0 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 6.77 (d, J = 9.0 Hz, 2H), 4.94 (s, 2H). HRMS (TOF ES+, m/z): calculated for $C_{16}H_{15}N_6O_4S$ ([M+H]$^+$) 387.0876; found 387.0879.

To a stirred solution of **20** (125 mg, 0.25 mmol) in THF (1 mL) was added 1.0 M solution of TBAF in THF (0.8 mL, 0.80 mmol) slowly at 0 °C under nitrogen atmosphere. After stirring at room temperature for 30 min, the reaction mixture was poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by preparative TLC plate (1:7 MeOH/$CHCl_3$) to give **16** (75.0 mg, 77%) as a white solid. ^{1}H NMR (DMSO-d_6) δ: 9.85 (s, 1H), 8.71 (s, 2H), 7.83 (s, 2H), 7.76 (d, J = 8.0 Hz, 2H), 7.19 (s, 1H), 6.96 (d, J = 7.5 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 6.77 (d, J = 8.5 Hz, 2H), 4.94 (s, 2H). HRMS (TOF ES+, m/z): calculated for $C_{16}H_{15}N_6O_4S$ ([M+H]$^+$) 387.0876; found 387.0858.

*2.2.22. 4-((3-(tert-Butyldimethylsilyloxy)-4-hydroxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**17**) and 4-((4-(tert-butyldimethylsilyloxy)-3-hydroxybenzyl)(4H-1,2,4-triazol-4-yl)amino)benzonitrile (**18**)*

To a stirred solution of **13** (1.50 g, 2.94 mmol) in EtOH (10 mL) and THF (12 mL) was added 10% Pd/C (150 mg). The suspension was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight. The catalyst was removed by filtration through Celite. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:30 MeOH/CH_2Cl_2) to give a mixture of two regioisomers **17** and **18** (750 mg, 61%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 9.22, 9.13 (s, 1H), 8.66, 8.62 (s, 2H), 7.76, 7.74 (d, J = 9.0 Hz, 2H), 6.78–6.56 (m, 5H), 4.88, 4.86 (s, 2H), 0.93, 0.92 (s, 9H), 0.12, 0.09 (s, 6H). HRMS (TOF ES+, m/z): calculated for $C_{22}H_{28}N_5O_2Si$ ([M+H]$^+$) 422.2012; found 422.2029.

*2.2.23. 2-(tert-Butyldimethylsilyloxy)-4-(((4-cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (**19**) and 2-(tert-butyldimethylsilyloxy)-5-(((4-cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)phenyl sulfamate (**20**)*

To a stirred solution of a mixture of two regioisomers **17** and **18** (600 mg, 1.42 mmol) in DMA (5 mL) was added sulfamoyl chloride (490 mg, 4.27 mmol) at 0 °C under nitrogen atmosphere. After stirring at room temperature overnight, the mixture was poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography on silica gel (1:10 MeOH/$CHCl_3$) to give two separated regioisomers **19** (159 mg, 22%) as a white solid and **20** (177 mg, 25%) as a white solid. Compound **19**, m.p. 138–140 °C. ^{1}H NMR (DMSO-d_6) δ: 8.75 (s, 2H), 7.97 (s, 2H), 7.77 (d, J = 9.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 1H), 6.98 (dd, J = 8.0, 1.5 Hz, 1H), 6.85 (d, J = 1.5 Hz, 1H), 6.76 (d, J = 9.0 Hz, 2H), 5.03 (s, 2H), 0.94(s, 9H), 0.12 (s, 6H). HRMS (TOF ES+, m/z): calculated for $C_{22}H_{29}N_6O_4SSi$ ([M+H]$^+$) 501.1740; found 501.1730. Compound **20**, m.p. 160–163 °C. ^{1}H NMR (DMSO-d_6) δ: 8.81 (s, 2H), 7.96 (s, 2H), 7.77 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 2.0 Hz, 1H), 7.05 (dd, J = 8.0, 2.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.77 (d, J = 9.0 Hz, 2H), 4.99 (s, 2H), 0.95(s, 9H), 0.16 (s, 6H). HRMS (TOF ES+, m/z): calculated for $C_{22}H_{29}N_6O_4SSi$ ([M+H]$^+$) 501.1740; found 501.1736.

*2.2.24. 4-(((4-Cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-hydroxyphenyl sulfamate (**21**)*

To a stirred solution of **19** (100 mg, 0.20 mmol) in THF (1 mL) was added 1.0 M solution of tetra-n-butylammonium fluoride in THF (0.8 mL, 0.80 mmol) slowly at 0 °C under nitrogen atmosphere. After stirring at room temperature for 1 h, the reaction mixture was poured into ice-water and extracted with EtOAc. The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the crude product was purified by preparative TLC plate (1:7 MeOH/$CHCl_3$) to give

21 (54 mg, 55%) as a white solid, m.p. 85–88 °C. ^{1}H NMR (DMSO-d_6) δ: 8.80 (s, 2H), 7.76 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 7.5 Hz, 1H), 6.87 (s, 1H), 6.79 (d, J = 7.5 Hz, 1H), 6.73 (d, J = 8.5 Hz, 2H), 5.00 (s, 2H). HRMS (TOF ES+, m/z): calculated for $C_{16}H_{15}N_6O_4S$ ([M+H]$^+$) 387.0876; found 387.0878.

2.3. Synthesis of carbon-11-labeled sulfamate derivatives

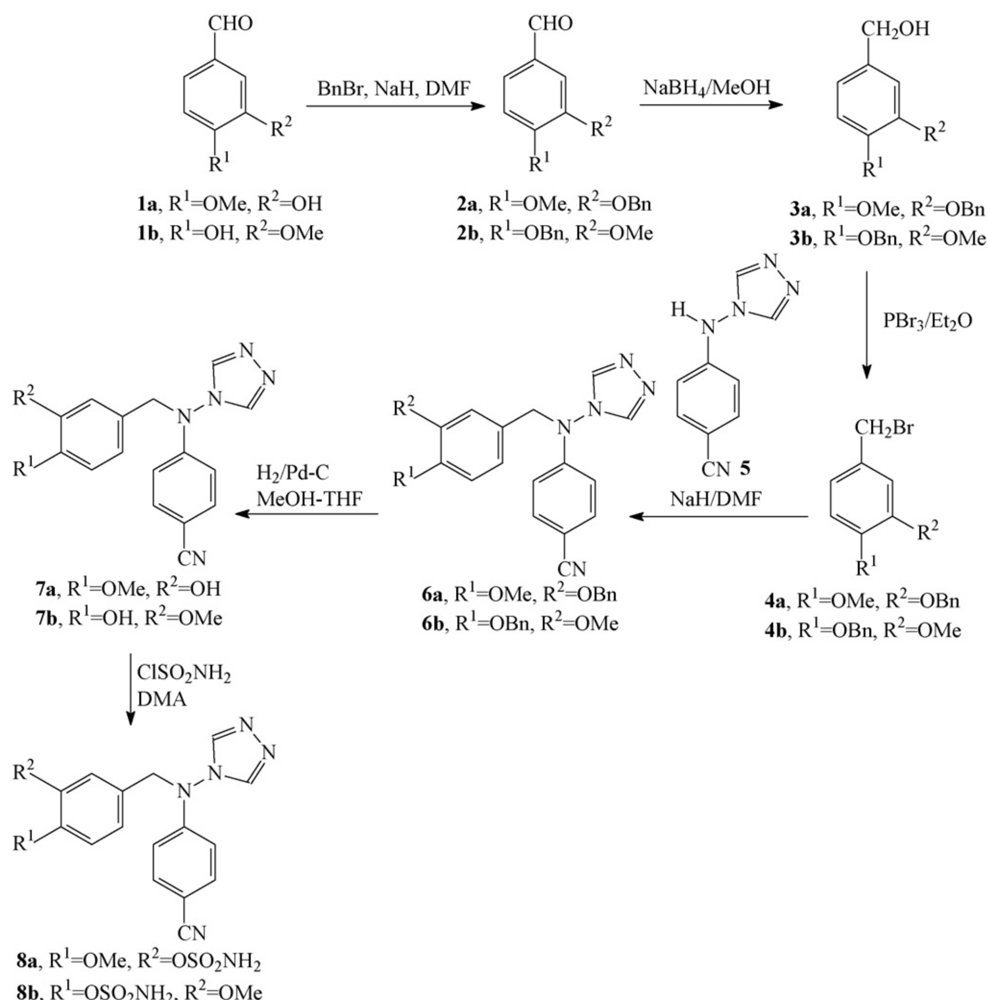
*2.3.1. General procedure for preparation of target tracers, 5-(((4-cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8a**) and 4-(((4-cyanophenyl)(4H-1,2,4-triazol-4-yl)amino)methyl)-2-[^{11}C]methoxyphenyl sulfamate ([^{11}C]**8b**)*

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). In a small reaction vial (5 mL), the precursor **16** or **21** (0.1 mg) was dissolved in CH_3CN (300 μL). To this solution was added 2N NaOH (2 μL). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method [18] 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 room temperature, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and reacted at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 2 mL injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product, [^{11}C]**8a** or [^{11}C]**8b**, was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 20–25 min from end of bombardment (EOB). Retention times in the analytical HPLC system were: t_R **16** = 3.50 min, t_R **8a** = 6.53 min, t_R [^{11}C]**8a** = 6.53 min; and t_R **21** = 3.45 min, t_R **8b** = 6.77 min, t_R [^{11}C]**8b** = 6.77 min. Retention times in the semi-preparative HPLC system were: t_R **16** = 5.70 min, t_R **8a** = 8.13 min, t_R [^{11}C]**8a** = 8.13 min; and t_R **21** = 5.58 min, t_R **8b** = 8.38 min, t_R [^{11}C]**8b** = 8.38 min. The radiochemical yields were 30–45% decay corrected to EOB, based on [^{11}C]CO_2.

3. Results and discussion

3.1. Chemistry

The target compounds sulfamate derivatives **8a** (IC_{50} 12 ± 1.9 nM for aromatase and >10,000 nM for STS) and **8b** (IC_{50} 42 ± 1 nM for aromatase and 380 ± 31 nM for STS) are potent DASSIs with nanomolar IC_{50} values [11] and *O*-methyl position amenable to labeling with carbon-11. Compounds **8a** and **8b** were prepared as shown in Scheme 1 using the literature method [11] with slight modifications. The improvements included modified syn-



Scheme 1. Synthesis of sulfamate derivative standards **8a** and **8b**.

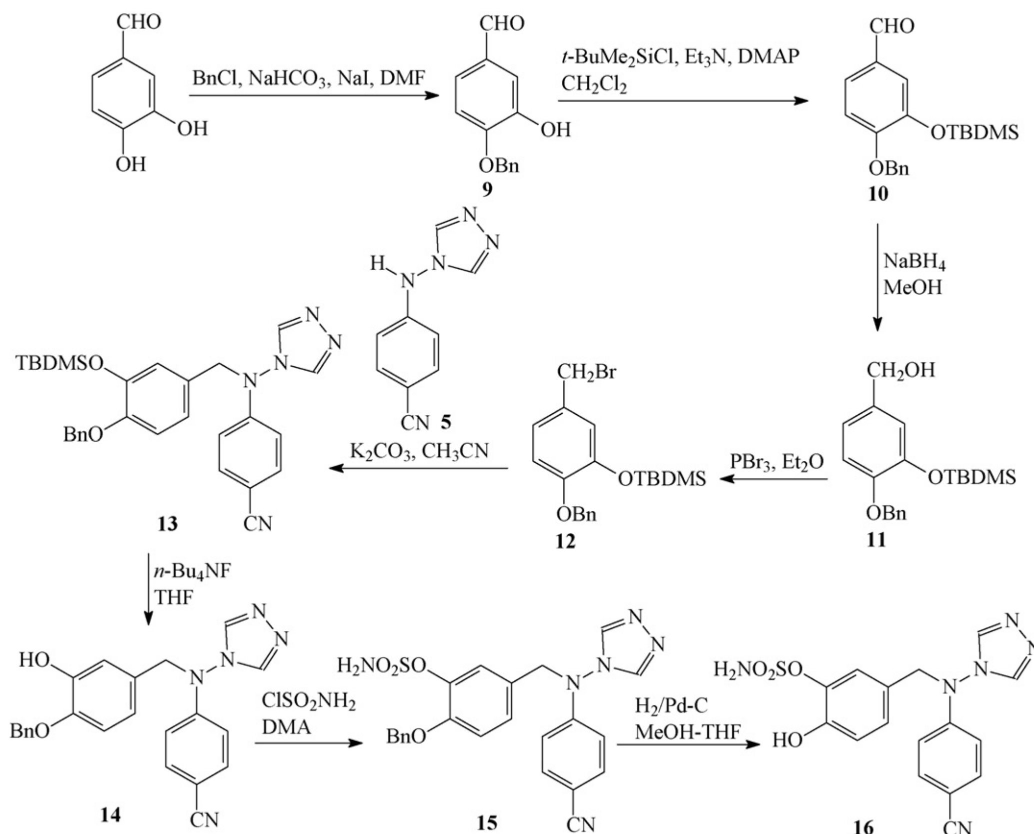
thetic approaches with moderate to excellent chemical yields, more complete experimental procedures and detailed spectral data. Benzylation of the hydroxyl group of benzaldehydes **1a** and **1b** with benzyl bromide in DMF using NaH as a base gave **2a** and **2b** in 83 and 77% yield, respectively [19,20]. Reduction **2a** and **2b** with $NaBH_4$ in MeOH yielded the alcohols **3a** and **3b** in 93 and 97% yield, respectively, followed by bromination with PBr_3 in Et_2O to afford the corresponding benzyl bromides **4a** and **4b** [23] in 93 and 94% yield, respectively. Coupling of the protected hydroxybenzyl bromides **4a** and **4b** and 4-((4-cyanophenyl)amino)-4*H*-1,2,4-triazole **5**, which was synthesized from 4-fluorobenzonitrile and 4-amino-4*H*-1,2,4-triazole using the established literature method [21] in 42% yield, in DMF with NaH as a base gave the tertiary amines **6a** and **6b** in 78 and 40% yield, respectively. Debenzylation of amines **6a** and **6b** by catalytic hydrogenation provided the resulting phenols **7a** and **7b** in 71 and 72% yield, respectively. Phenols **7a** and **7b** were sulfamoylated with an excess of sulfamoyl chloride in DMA to yield the sulfamates **8a** and **8b** in 74 and 83% yield, respectively.

In order to obtain phenolic precursors, we envisioned **16** and **21** could be prepared by *O*-demethylation of compounds **8a**–**b**. A variety of protocols [24] were screened for this purpose including protic acid (HBr), Lewis acids (BBr_3, $AlCl_3$/EtSH, LiCl/DMF), and base (EtSNa). However, in all cases, the demethylated product was obtained with very low yield. As a result of these unsuccessful attempts, an alternative strategy was first explored to synthesize versatile intermediate with differentiated protected hydroxyl group for further elaboration. As depicted in Scheme 2, selective benzylation of the more acidic 4-hydroxyl group was performed by the treatment of 3,4-dihydroxybenzaldehyde with benzyl chloride in DMF using $NaHCO_3$ as a base and a catalytic amount of NaI to give compound **9** in 70% yield [22]. Protection of the 3-hydroxyl group of **9** to its TBDMS (*tert*-butyldimethylsilyl) ether with *tert*-butyldimethylsilyl chloride, triethylamine and DMAP in CH_2Cl_2 afforded compound **10** in 97% yield [25]. The aldehyde **10** was reduced to the alcohol **11** with $NaBH_4$ in MeOH in 85% yield and subsequently converted to the benzyl bromide **12** with PBr_3 in Et_2O in 72% yield. Coupling of the protected hydroxybenzyl bromide **12** with the triazole **5** in CH_3CN with K_2CO_3 as a base provided the tertiary amine **13** in 46% yield. Desilylation of the amine **13** with TBAF in THF obtained the phenol **14** in 91% yield. Sulfamoylation of the phenol **14** with sulfamoyl chloride in DMA afforded the sulfamate **15** in 84% yield, which in turn was debenzylated by catalytic hydrogenation to obtain the free phenol **16** in 17% yield.

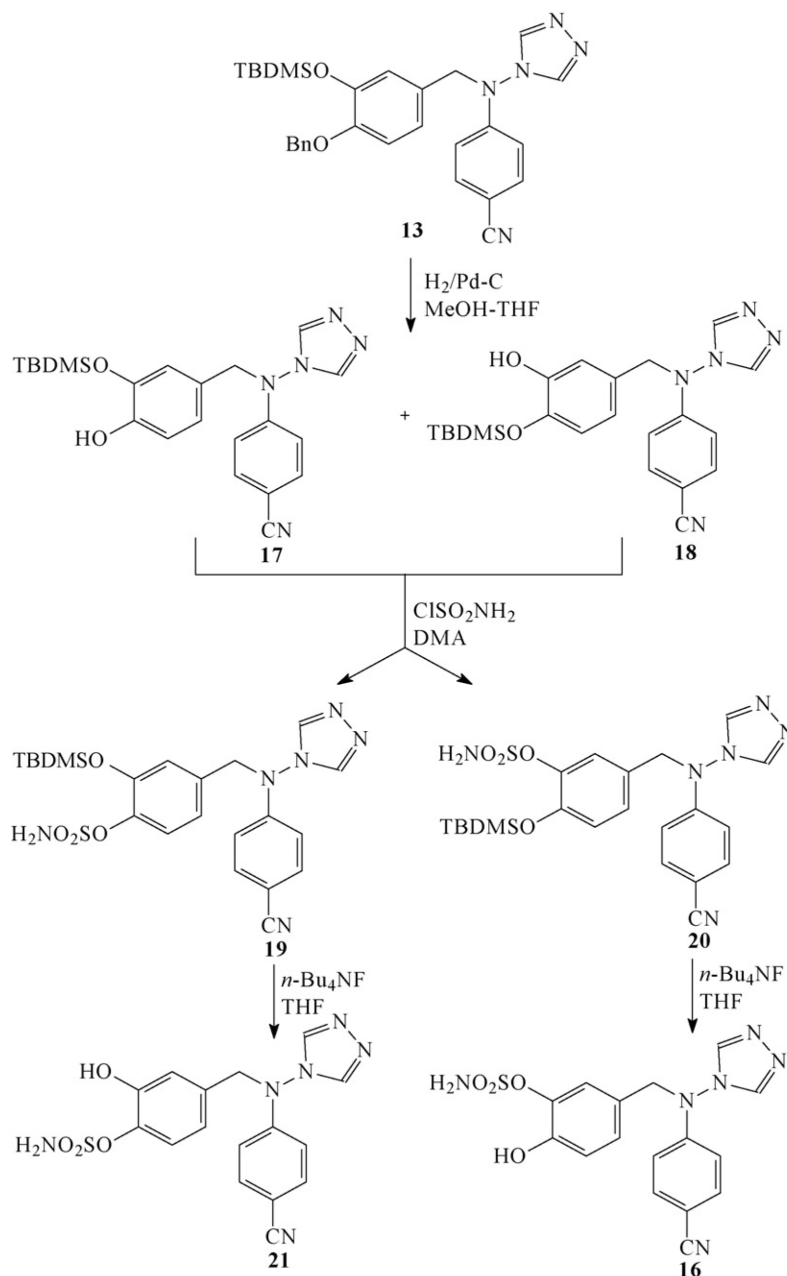
Contrary to the sequence of selective removal of the protected group for **16**, as indicated in Scheme 3, de-protection of the benzyl group of the amine **13** by catalytic hydrogenation afforded a mixture of two regioisomers **17** and **18** in 61% yield, which was difficult to separate and purify. The ratio of the resulting regioisomers **17** and **18** was determined by ^1H NMR as 1:1, this indicated that **18** is the product of 1,2-*O*,*O*-TBDMS migration of **17** [26]. Without isolation, the mixture of **17** and **18** was sulfamoylated in DMA to give sulfamate regioisomers **19** and **20**. Compounds **19** and **20** were readily separated by silica gel column chromatography in 22 and 25% yield, respectively. Desilylation of **19** and **20** with TBAF in THF gave precursors **21** and **16** in 55 and 77% yield, respectively.

3.2. Radiochemistry

Synthesis of target radiotracers $[^{11}C]$**8a** and $[^{11}C]$**8b** is indicated in Scheme 4. Precursor **16** or **21** was labeled by a reactive $[^{11}C]$methylating agent, $[^{11}C]CH_3OTf$ [18,27] prepared from $[^{11}C]CO_2$, in the presence of 2N NaOH in acetonitrile through the *O*-$[^{11}C]$methylation and isolated by semi-preparative reversed-phase HPLC method to provide target tracer $[^{11}C]$**8a** or $[^{11}C]$**8b** in 30–45% radiochemical yields, decay corrected to EOB, based on $[^{11}C]CO_2$.



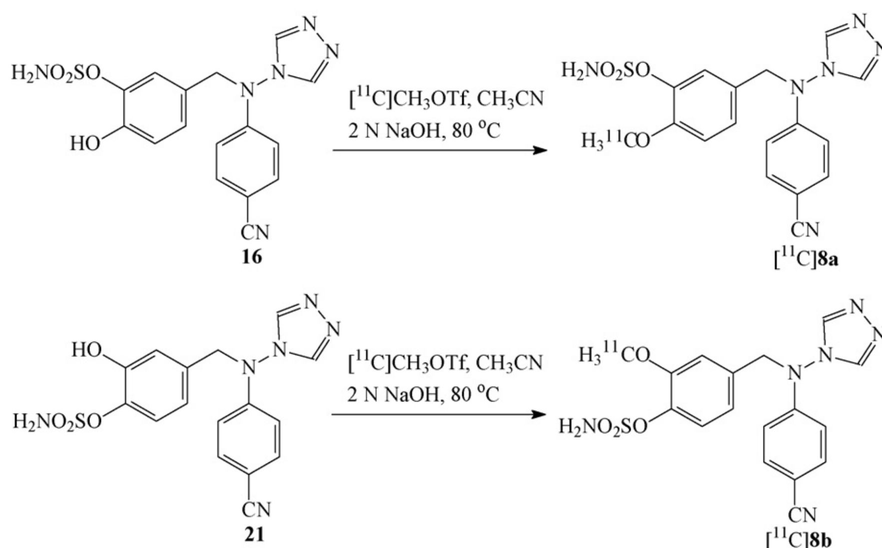
Scheme 2. Synthesis of sulfamate derivative precursor **16**.



Scheme 3. Synthesis of sulfamate derivative precursors **21** and **16**.

The synthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis [28,29]. The specific activity of [^{11}C]**8a** and [^{11}C]**8b** was in a range of 222–370 GBq/μmol at EOB measured by the on-the-fly technique using semi-preparative HPLC method during synthesis [29] and 111–185 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC method [30], respectively. Chemical purity and radiochemical purity were determined by analytical HPLC method [30]. The chemical purity of the precursors **16** and **21**, and reference standards **8a** and **8b** was >98%. The radiochemical purity of the target tracers [^{11}C]**8a** and [^{11}C]**8b** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracers [^{11}C]**8a** and [^{11}C]**8b** was >96% determined by reversed-phase HPLC through UV flow detector.

The precursors have the potential to methylate either at the nitrogen or at the oxygen position. The radio-HPLC chromatogram of the radiosynthesis did show other radiolabeled minor by-products, because the methylation of the precursors at the nitrogen position is a potential competing reaction. However, the evidence provided by the radio-HPLC chromatogram of the radiosynthesis showed that the precursors are mainly methylated at the oxygen position to produce the major target radiotracers compared to the retention times of the reference standards. These results are consistent with the theoretical explanation that the deprotonization at the oxygen position of the precursor is easier than at the nitrogen position under basic conditions (2N NaOH) since the acidity of HO– of the precursor is greater than the acidity of H_2NO_2SO– of the precursor, and the methylation of the precursor will prefer to occur at the oxygen position rather than at the nitrogen position. More strong basic conditions such as NaH may increase the yield of the methylated product at the nitrogen position [15]. Compared to the acidic HO– of the precursor, H_2NO_2SO– of the precursor tends to be basic.



Scheme 4. Synthesis of carbon-11-labeled sulfamate derivatives [11C]**8a** and [11C]**8b**.

3.3. Conclusions

In summary, an efficient and convenient synthesis of new carbon-11-labeled sulfamate derivatives, has been well developed. The synthetic methodology employed classical organic chemistry such as selective benzylation and debenzylation, reduction, bromination, coupling reaction, protecting and de-protecting reactions, and sulfamoylation to prepare a series of new sulfamate derivative precursors and standard compounds. The target radiotracers were prepared by the *O*-[11C]methylation of their corresponding phenolic precursors using a reactive [11C]methylating agent, [11C]CH_3OTf, and isolated by a HPLC procedure in high radiochemical yields, short overall synthesis time, and great specific radioactivities. These chemistry results combined with the reported *in vitro* biological data [11] encourage further *in vivo* biological evaluation of new carbon-11-labeled DASSIs as candidate PET radiotracers for imaging of steroid biosynthetic enzymes aromatase and STS in breast cancer.

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