Strategies for the Labeling of Halogen-Substituted Peroxisome Proliferator-Activated Receptor γ Ligands: Potential Positron Emission Tomography and Single Photon Emission Computed…

ARTICLE *in* BIOCONJUGATE CHEMISTRY · MARCH 2007

Impact Factor: 4.51 · DOI: 10.1021/bc060191g · Source: PubMed

Strategies for the Labeling of Halogen-Substituted Peroxisome Proliferator-Activated Receptor γ Ligands: Potential Positron Emission Tomography and Single Photon Emission Computed Tomography Imaging Agents

Byung Chul Lee,[†] Kyo Chul Lee,[‡] Hsiaoju Lee,[§] Robert H. Mach,[§] and John A. Katzenellenbogen*[,†]

Department of Chemistry, University of Illinois, Urbana, Illinois 61801, Laboratory of Radiopharmaceuticals, Korea Institute of Radiological and Medical Sciences, Seoul, South Korea, and Division of Radiological Sciences, Washington University School of Medicine, St. Louis, Missouri 63110. Received June 30, 2006; Revised Manuscript Received January 12, 2007

Well-known as an important regulator of lipid metabolism and adipocyte differentiation, the peroxisome proliferator-activated receptor γ (PPARγ) also has potential use as a target for antitumor therapy in certain cancers. To develop agents for radionuclide imaging PPARγ in vivo, we synthesized fluorine, bromine, and iodine-substituted analogs (**1**−**3**) of a high-affinity benzophenone−tyrosine PPARγ ligand; all three analogs retain very high affinity for the PPARγ receptor. In preparation for the synthesis of these PPARγ ligands in radiolabeled form, we have synthesized two types of precursors: (a) an aryltributylstannane (**9**), from which the bromine and iodine-substituted analogs (**2** and **3**) can readily be prepared by electrophilic destannylation, and (b) three diaryliodonium tosylate derivatives (**12a**−**c**), precursors for nucleophilic aromatic fluorination using fluoride ion. Conditions were developed whereby the thiophenyliodonium tosylate (**12c**) underwent nucleophilic aromatic substitution with fluoride ion, efficiently and in short reaction times, to produce the desired fluorine-substituted target compound **1**. These reactions laid the groundwork for producing these three PPARγ ligands in radiolabeled form; in addition, our use of diaryliodonium ion precursors for aromatic fluorination in this series provides an example that should encourage application of this approach for radiofluorination of more complicated radiopharmaceuticals.

INTRODUCTION

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily, of which three subtypes are known: PPARα, PPARγ, and PPARδ (*1*−*3*). Each of these subtypes appears to regulate specific aspects of lipid metabolism, glucose homeostasis, inflammation, and cell differentiation (*4*−*6*). PPARγ can also play a critical role in certain cancers (*6*−*15*), and PPARγ ligands can induce liposarcoma cells to stop proliferating, lose their malignant character, and undergo terminal differentiation to adipocytes. In fact, in clinical trials, the PPARγ ligand troglitazone has been shown to induce differentiation of liposarcoma solid tumors in patients with advanced disease (*12*, *14*). PPARγ is also expressed in several breast cancer cell lines and in animal tumor models for breast cancer derived from primary and metastatic breast tumors, and PPARγ activation may alter the growth characteristics of breast cancer cells (*11*, *15*). Thus, PPARγ is an important target for in vivo imaging using positron emission tomography (PET) or single photon emission computed tomography (SPECT) that might be useful in assessing lipid metabolism disorders in obesity, type-2 diabetes, or vascular disease (*16*) or in identifying small metastatic tumors.

With this goal in mind, some time ago our group evaluated as PET imaging agents a set of fluorine-18 labeled PPARγ receptor ligands, based on the known compound SB 213068 (Scheme 1) (*17*). The binding of these fluorine-substituted compounds was selective for PPARγ compared to the PPARα and PPARδ subtypes; however, their affinity for PPARγ, though good, was not in the low nanomolar region as would be desirable for in vivo imaging. Also, one SB 213068 analogue (**5**), prepared in the fluorine-18 labeled form (*17*), did not show evidence of receptor-mediated uptake in tissue distribution studies in brown fat, the most receptor-rich tissue (*18*).

Since then, however, there have been a number of significant advances in the development of PPAR ligands, most notably, the discovery of systems that have much higher affinity and selectivity for both PPARγ and PPARα, largely through efforts of researchers at Glaxo SmithKline (GSK) (*19*−*22*). In particular, members of the novel benzophenone−tyrosine class (farglitazar GI 262570, **4,** Scheme 1) have particularly high affinity for PPARγ, and they show high potency in vivo. In addition, the use of the high-affinity enantiomer of this chiral compound increases its receptor binding affinity and would likely also enhance its target tissue uptake selectivity (*19*, *23*, *24*). Thus, we have selected farglitazar as a parent compound for the development of radiohalogen-labeled PPARγ-based imaging agents.

In this investigation, we have prepared three farglitazar analogues that are substituted at a tolerant aromatic ring position with fluorine, bromine, or iodine (**1**−**3**), which could be labeled, respectively, with fluorine-18, bromine-76, and iodine-124 for PET imaging or with other radionuclides for SPECT imaging. All three farglitazar derivatives retain high binding affinity for PPARγ. In model procedures for radiolabeling, we developed methods by which these three compounds can be produced from suitable precursors by reaction with simple halide species. The bromine and iodine-substituted compounds (**2** and **3**) were readily produced by electrophilic aromatic destannylation of a trialkyltin precursor. The use of electrophilic aromatic halogenation is not suitable for fluorine-18 labeling at the high specific activity needed for imaging receptors, which generally needs to be in the range of 1000 Ci/mmol for nuclear receptors such

* Corresponding author. John A. Katzenellenbogen, Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801, Telephone: 217-333-6310. Fax: 217-333-7325. E-mail: jkatzene@uiuc.edu.

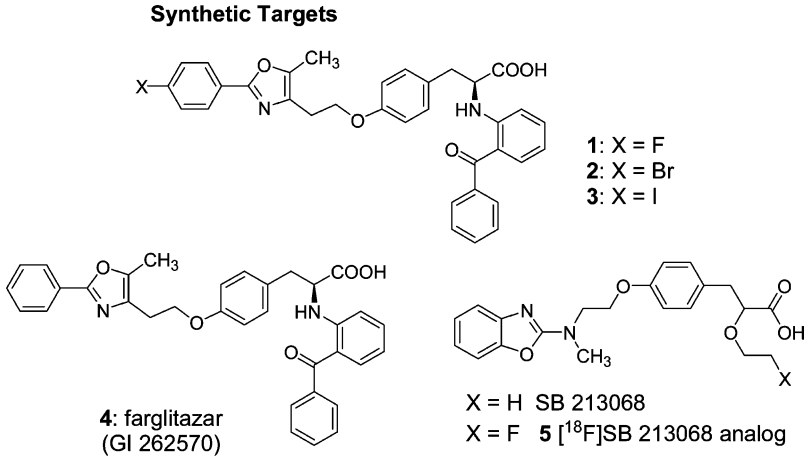
[†] University of Illinois.

[‡] Korea Institute of Radiological and Medical Sciences.

[§] Washington University School of Medicine.

10.1021/bc060191g CCC: $37.00
Published on Web 02/23/2007

Scheme 1. Structure of Synthetic Targets and Known PPARγ Ligands in This Study



as PPARγ, because they are present at only nanomolar concentrations in target tissues (*25*). Preparation of fluorine-18 labeled radiopharmaceuticals at this specific activity level requires the use of fluoride ion as the reagent. The aromatic ring position in question is also not sufficiently activated for conventional nucleophilic aromatic substitution reactions with fluoride ion. To overcome this problem, we synthesized iodonium salts from the same trialkyltin intermediate using various hydroxyl(tosyloxy)iodoarenes (*26*−*29*). Conditions were then optimized to produce the desired fluorine-substituted compound by nucleophilic aromatic substitution by fluoride ion on this highly activated system.

The methods we have developed here lay the groundwork for the synthesis of these three halogen-substituted PPARγ ligands in iodine, bromine, and fluorine radiolabeled form. The last method, using diaryliodonium salts for fluorine labeling, in particular, demonstrates an approach that is effective for introducing fluoride ion into aromatic rings that are not sufficiently activated for conventional nucleophilic aromatic substitution reactions, and it illustrates that this approach should be applicable for the preparation of other complex fluorine-18 labeled radiopharmaceuticals.

EXPERIMENTAL SECTION

Materials and Methods. Solvents and reagents were purchased from the following commercial sources: Aldrich, Fisher, or Acros. Anhydrous THF, Et_2O, and CH_2Cl_2 were collected using a solvent dispensing system built by J. C. Meyer based on a design developed by Pahgborn (*30*). TLC was performed on Merck F_{254} silica plates. The chemical shifts were reported in parts per million downfield from TMA and were referenced to the internal solvent peaks. Coupling constants were reported in hertz. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian U400 and U500 Instruments (Palo Alto, CA). Melting points were checked using a Thomas-Hoover capillary melting point apparatus (Philadelphia, PA) and are uncorrected. Low-resolution mass spectra (MS) were recorded on a Micromass 70-VSE, Finnigan MAT CH-5, and ZAB-SE spectrometers, respectively. High-resolution mass spectra were obtained on a VG 70-SE-4E spectrometer. Mass spectra were acquired in the positive ion mode under electron impact (EI, 70 eV), fast-atom bombardment mass (FAB), and electrospray ionization (ESI) methods. Methyl 4-bromo-3-oxopentanoate and (2*S*)-2-((2-benzoylphenyl)amino)-3-(4-hydroxyphenyl)-propionic acid methyl ester were prepared according to literature methods (*19*, *20*). Koser's reagent **11a** was purchased from Aldrich. Compounds **10a**, **10b**, and **11c** were prepared according to the literature method (*31*−*33*): Diacetoxyiodo-*p*-methoxybenzene (**10a**) yield 69%, mp 131−135 °C, literature (*31*) mp 133−135 °C; 2-(diacetoxyiodo)thiophene (**10b**) yield 51%, mp 119−125 °C; literature (*32*) mp 120−122 °C (decomp); 2-[hydroxy(tosyloxy)-iodo]thiophene (**11c**) yield 95%, mp 62−66 °C (decomp), literature (*33*) mp 65−68 °C (decomp).

[2-(4-Fluorophenyl)-5-methyloxazol-4-yl]-acetic Acid Methyl Ester (6a). To a solution of 4-fluorobenzamide (1.23 g, 8.85 mmol) in 1,2-dichlorobenzene (50 mL) was added methyl 4-bromo-3-oxopentanoate (1.84 g, 8.85 mmol). The reaction mixture was refluxed using a Dean−Stark trap at 120 °C for 16 h. The resulting dark slurry was cooled to room temperature, diluted with EtOAc (100 mL), and washed with aqueous sodium bicarbonate (100 mL). The organic layer was separated and dried with sodium sulfate, and the solvents were removed in vacuo. Purification of the material by silica gel flash column chromatography using 10% EtOAc/ hexane as eluant afforded the product **6a** (683 mg, 31%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.56 (s, 2H), 3.73 (s, 3H), 7.10 (m, 2H), 7.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.25, 31.91, 52.19, 115.77 (J = 22.0 Hz), 123.90 (J = 4.0 Hz), 128.09 (J = 8.4 Hz), 129.35, 145.72, 158.76, 163.77 (J = 249.6 Hz), 170.72; MS (FAB) *m*/*z* 250 (M + H). HRMS calcd for $C_{13}H_{13}FNO_3$ 250.0879, found 250.0878. Registry Number: 196810-28-3.

[2-(4-Bromophenyl)-5-methyloxazol-4-yl]acetic Acid Methyl Ester (6b). Prepared from 4-bromobenzamide by the method used to prepare compound **6a** and afforded the title compound **6b** (35%) as a white solid: mp 58−61 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.33 (s, 3H), 3.55 (s, 2H), 3.71 (s, 3H), 7.52−7.54 (m, 2H), 7.81−7.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.22, 31.84, 52.16, 124.20, 126.41, 127.42, 129.60, 131.82, 146.00, 158.64, 170.66; MS (ESI) *m*/*z* 310 (M^+). HRMS calcd for $C_{13}H_{13}BrNO_3$ 310.0079, found 310.0082. Registry N591775-29-0.

[2-(4-Iodophenyl)-5-methyloxazol-4-yl]acetic Acid Methyl Ester (6c). Prepared from 4-iodobenzamide by the method used to prepare compound **6a** and afforded the title compound **6c** (37%) as a white solid: mp 90−91 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.56 (s, 2H), 3.73 (s, 3H), 7.69−7.71 (m, 2H), 7.75−7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.29, 31.90, 52.22, 96.21, 127.00, 127.52, 129.65, 137.81, 146.09, 158.82, 170.72; MS (ESI) *m*/*z* 358 (M^+). HRMS calcd for $C_{13}H_{13}INO_3$ 357.9940, found 357.9940.

2-[2-(4-Fluorophenyl)-5-methyloxazol-4-yl]ethanol (7a). To a stirred solution of compound **6a** (612 mg, 2.46 mmol) in tetrahydrofuran (10 mL) at 0 °C was added 1.0 M solution of $LiAlH_4$ (2.46 mL, 2.46 mmol) in tetrahydrofuran. The resulting solution was stirred at room temperature for 45 min, then cooled to 0 °C, and quenched by careful addition of a minimum volume of H_2O. The resulting heterogeneous mixture was filtered under vacuum, and the filtrate was concentrated in vacuo. Purification

of this material by silica gel flash column chromatography using 40% EtOAc/hexane as eluant afforded the product **7a** (326 mg, 60%) as a pale yellow solid: mp 102−104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.72 (t, J = 6.0 Hz, 2H), 3.41 (br, 1H), 3.92 (t, J = 6.0 Hz, 2H), 7.10−7.14 (m, 2H), 7.96−7.99 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.10, 27.90, 61.77, 115.90 (J = 22.0 Hz), 128.15 (J = 4.0 Hz), 129.35 (J = 8.4 Hz), 133.66, 144.26, 158.69, 165.11 (J = 249.6 Hz); MS (EI) m/z 221 (M^+). HRMS calcd for $C_{12}H_{12}FNO_2$ 221.0852, found 221.0853. Registry Number: 853309-24-7.

2-[2-(4-Bromophenyl)-5-methyloxazol-4-yl]ethanol (7b). To a stirred solution of compound **6b** (465 mg, 1.5 mmol) in methanol (10 mL) at room temperature was added $NaBH_4$ (570 mg, 15.0 mmol) carefully and portionwise over a 3 h period using a spatula. The resulting solution was stirred at room temperature for 6 h, then cooled to 0 °C and quenched by careful addition of H_2O. Methanol was evaporated and diluted with EtOAc (15 mL) and brine (15 mL). The organic layer was separated and dried with sodium sulfate, and the solvents were removed in vacuo. Purification of the material by silica gel flash column chromatography using 40% EtOAc/hexane as eluant afforded the product **7b** (318 mg, 75%) as a white solid: mp 85−91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 2.70 (t, J = 6 Hz, 2H), 3.23 (br, 1H), 3.89 (t, J = 6.0 Hz, 2H), 7.65−7.68 (m, 2H), 7.73−7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.06, 28.12, 61.67, 124.23, 126.29, 127.34, 131.88, 134.07, 144.51, 158.58; MS (EI) m/z 283 (M^+). HRMS calcd for $C_{12}H_{12}BrNO_2$, 283.0031 found 283.0037. Registry Number: 328918-84-9.

2-[2-(4-Iodophenyl)-5-methyloxazol-4-yl]ethanol (7c). Prepared from compound **6c** by the method used to prepare compound **7b** and afforded the title compound **7c** (62%) as a white solid: mp 108−111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H), 2.70 (t, J = 6.0 Hz, 2H), 3.23 (br, 1H), 3.89 (t, J = 6.0 Hz, 2H), 7.65−7.68 (m, 2H), 7.73−7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.09, 28.10, 61.68, 96.18, 126.81, 127.38, 134.07, 137.81, 144.56, 158.70; MS (EI) m/z 328 (M^+). HRMS calcd for $C_{13}H_{13}INO_3$ 328.9912, found 328.9907.

(2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-fluorophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid Methyl Ester (8a). To a stirred solution of triphenylphosphine (165 mg, 0.49 mmol) and diethyl azodicarboxylate (77 μL, 0.49 mmol) in tetrahydrofuran (5 mL) at 0 °C was added the mixture of (2*S*)-2-((2-benzoylphenyl)amino)-3-(4-hydroxyphenyl)propionic acid methyl ester (183 mg, 0.49 mmol) and compound **7a** (108 mg, 0.49 mmol) in tetrahydrofuran (2 mL) under nitrogen atmosphere. After the mixture was stirred at 0 °C for 1 h, the resulting solution was stirred for 8 h at room temperature under nitrogen. The solvent was removed in vacuo. The crude reaction mixture was purified by silica gel flash column chromatography using 30% EtOAc/hexane as eluant to afford the product **8a** (140 mg, 55%) as a yellow oil (55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.94 (t, J = 6.8, 2H), 3.11 (dd, J = 13.6, 7.2 Hz, 1H), 3.20 (dd, J = 14.0, 6.0 Hz, 1H), 3.69 (s, 3H), 4.19 (t. J = 6.4 Hz, 2H), 4.38 (m, 1H), 6.56−6.64 (m, 2H), 6.82 (d, J = 8.0 Hz, 2H), 7.09 (m, 2H), 7.17 (d, J = 8.0 Hz, 2H), 7.33 (m, 1H), 7.42−7.53 (m, 4H), 7.60 (d, J = 4.0 Hz, 2H), 7.95−7.98 (m, 2H), 8.91 (d, J = 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.18, 26.28, 38.06, 52.21, 57.96, 66.52, 111.73, 114.62, 114.95, 115.79 (J = 22. 0 Hz), 118.19, 124.19 (J = 3.6 Hz), 127.95 (J = 8.3 Hz), 128.04, 128.38, 129.09, 130.29, 130.89, 132.71, 134.91, 135.55, 140.28, 145.10, 150.19, 157.80, 158.66, 163.69 (J = 249. 1 Hz), 173.05, 199.30; MS (FAB) m/z 579 (M + H). HRMS cald for $C_{35}H_{32}FN_2O_5$ 579.2295, found 579.2295.

(2*S*)-[2-Benzoylphenylamino]-3-{4-[2-(4-bromophenyloxazol 5-methyl-4-yl)ethoxy]phenyl}propionic Acid Methyl Ester (8b). Prepared from **7b** by the method used to prepare compound **8a** and obtained as a yellow oil (43%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (s, 3H), 2.94 (t, J = 6.5, 2H), 3.09−3.22 (m, 2H), 3.69 (s, 3H), 4.18 (t. J = 7.0 Hz, 2H), 4.37 (q, J = 13.5 Hz, 1H), 6.57 (t, J = 8.5 Hz, 1H), 6.63 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 7.31−7.34 (m, 1H), 7.42−7.60 (m, 8H), 7.83 (d, J = 8.5 Hz, 2H), 8.90 (d, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.21, 26.25, 38.01, 52.21, 57.91, 66.45, 111.70, 114.59, 114.93, 118.15, 124.03, 126.67, 127.36, 128.02, 128.36, 129.07, 130.27, 130.88, 131.87, 132.98, 134.90, 135.54, 140.24, 145.42, 150.16, 157.75, 158.55, 173.03, 199.28; MS (ESI) m/z 639 (M^+). HRMS calcd for $C_{35}H_{31}BrN_2O_5$ 639.1495, found 639.1487.

(2*S*)-[2-Benzoylphenylamino]-3-{4-[2-(4-iodophenyloxazol 5-methyl-4-yl)ethoxy]phenyl}propionic Acid Methyl Ester (8c). Prepared from **7c** by the method used to prepare compound **8a** and obtained as a yellow oil (50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.93 (t, J = 6.8, 2H), 3.09−3.22 (m, 2H), 3.69 (s, 3H), 4.18 (t. J = 6.4 Hz, 2H), 4.38 (q, J = 13.2 Hz, 1H), 6.55−6.59 (m, 1H), 6.63 (d, J = 8.8 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 7.31−7.35 (m, 1H), 7.42−7.53 (m, 4H), 7.58−7.60 (m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 8.91 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.18, 26.22, 37.98, 52.17, 57.87, 66.43, 95.92, 111.68, 114.57, 114.90, 118.13, 127.17, 127.37, 127.99, 128.33, 129.04, 130.25, 130.85, 132.97, 134.86, 135.49, 137.77, 140.21, 145.42, 150.12, 157.71, 158.60, 172.98, 199.22; MS (ESI) m/z 687 (M + H). HRMS calcd for $C_{35}H_{32}IN_2O_5$ 687.1356, found 687.1358.

(2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-fluorophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (1). A stirred solution of compound **8a** (50 mg, 0.086 mmol) in THF/MeOH (v/v = 3:1, 1.25 mL) was treated with 1.0 M LiOH (172 μL, 0.172 mmol). The resulting solution was stirred at room temperature for 4 h. To the reaction mixture was added 1 N HCl (200 uL), and that solution was extracted with EtOAc (5 mL). The organic layer was separated and dried with sodium sulfate. The obtained organic layers were concentrated and purified by silica gel flash column chromatography using 5% MeOH/CH_2Cl_2 as eluant to afford the product **1** (47 mg. 96%) as a yellow solid: mp 95−101 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 2.29 (s, 3H), 2.89 (t, J = 6.4 Hz, 2H), 3.15 (dd, J = 13.8, 7.4 Hz, 1H), 3.23 (dd, J = 13.8, 5 Hz, 1H), 4.14 (t, J = 6.4 Hz, 2H), 4.27−4.30 (m, 1H), 6.49 (t, J = 7.6 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.4 Hz, 2H), 7.15−7.22 (m, 4H), 7.29−7.35 (m 2H), 7.43−7.54 (m, 4H), 7.94−7.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.19, 26.15, 37.99, 57.90, 66.38, 111.67, 114.54, 114.90, 115.81 (J = 22.0 Hz), 118.09, 127.97, 128.01 (J = 8.0 Hz), 128.29, 129.03, 130.23, 130.85, 132.40, 134.88, 135.50, 140.17, 145.23, 150.09, 157.67, 158.61, 163.71 (J = 249.0 Hz), 172.99, 199.24; MS (FAB) m/z 565 (M + H). HRMS calcd for $C_{34}H_{30}FN_2O_5$ 565.2139, found 565.2139. Registry Number: 196808-49-8.

(2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-bromophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (2). Prepared from **8b** by the method used to prepare compound **1** and obtained as a yellow solid (98%): mp 102−107 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.27 (s, 3H), 2.82 (t, J = 6.5 Hz, 2H), 2.97 (dd, J = 13.5, 6.5 Hz, 1H), 3.16 (dd, J = 13.5, 5 Hz, 1H), 4.04 (t, J = 7.0 H, 2H), 4.21 (m, 1H), 6.43 (t, J = 7.0 Hz, 1H), 6.69−6.74 (m, 3H), 7.08 (d, J = 8.5 Hz, 2H), 7.24−7.25 (m, 2H), 7.46−7.55 (m, 5H), 7.66 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 8.77 (d, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.84, 25.53, 36.83, 58.06, 65.96, 112.60, 113.23, 113.94, 116.77, 123.42, 126.25, 127.35, 127.84, 128.16, 128.55, 130.45, 130.76, 132.09, 132.97, 134.59, 134.68, 140.21, 145.50,

150.30, 156.66, 157.49, 175.13, 197.59; MS (ESI) *m*/*z* 625(M + H). HRMS calcd for $C_{34}H_{30}BrN_2O_5$ 625.1338, found 625.1335.

(2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-iodophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (3). Prepared from **8c** by the method used to prepare compound **1** and obtained as a yellow solid (98%): mp 107−110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.29 (s, 3H), 2.85 (t, J = 6.5 Hz, 2H), 2.98 (dd, J = 13.5, 6.5 Hz, 1H), 3.13 (dd, J = 13.5, 5 Hz, 1H), 4.10 (t, J = 7.0 H, 2H), 4.42 (m, 1H), 6.54 (t, J = 7.5 Hz, 1H), 6.76 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 1H), 7.09 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.0 Hz, 1H), 7.37 (t, J = 7.5 Hz, 1H), 7.47−7.51 (m, 4H), 7.54−7.57 (m 1H), 7.65 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.5 Hz, 2H), 8.68 (d, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 9.84, 25.50, 36.58, 56.80, 65.96, 96.96, 112.47, 114.14, 117.08, 126.49, 127.23, 128.20, 128.61, 129.18, 130.36, 130.94, 132.92, 134.68, 134.92, 137.88, 139.92, 145.47, 149.91, 156.93, 157.70, 162.30, 173.62, 197.99; MS (ESI) *m*/*z* 673 (M + H), 515 (80%), 257 (100%). HRMS calcd for $C_{34}H_{30}IN_2O_5$ 673.1199, found 673.1190.

(2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-tributylstannanyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid Methyl Ester (9). To a solution of tetrakis(triphenylphospine)-palladium(0) (22 mg, 5 mol %) and bis(tributyltin) (217 μL, 0.418 mmol) in dry toluene (5 mL) was added (2*S*)-[2-benzoylphenylamino]-3-{4-[2-(4-iodophenyloxazol 5-methyl-4-yl)ethoxy]phenyl}propionic acid methyl ester **8c** (258 mg, 0.38 mmol) in dry toluene (3 mL) at room temperature under nitrogen atmosphere. After addition, the reaction mixture was heated to 120 °C for 6 h. After evaporation of the solvent, the concentrate was filtered through a layer of Celite, and the residue was subjected to flash chromatography using 20% EtOAc/hexane as eluant to afford the product **9** (271 mg, 84%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 7.2 Hz, 9H), 1.08 (t, J = 8.2, 6H), 1.30−1.36 (m, 6H), 1.50−1.58 (m, 6H), 2.35 (s, 3H), 2.95 (t, J = 6.4 Hz, 2H), 3.09−3.22 (m, 2H), 3.69 (s, 3H), 4.19 (t, J = 6.8 Hz, 2H), 4.38 (q, J = 13.2 Hz, 1H), 6.57 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 7.31−7.35 (m, 1H), 7.42−7.61 (m, 8H), 7.89 (d, J = 8.4 Hz, 2H), 8.91 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.58, 10.23, 13.66, 26.33, 27.31, 29.03, 38.09, 52.22, 57.97, 66.61, 111.72, 114.61, 114.91, 118.16, 124.95, 127.19, 128.01, 128.29, 129.07, 130.24, 130.86, 132.58, 134.87, 135.51, 136.66, 140.23, 144.83, 144.97, 150.15, 157.79, 159.73, 173.02, 199.22; MS (ESI) *m*/*z* 851 (M + H, 10%), 625 (10%), 291 (80%), 235 (100%). HRMS calcd for $C_{47}H_{59}N_2O_5Sn$ 851.3446, found 851.3486.

[(Hydroxy)(tosyloxy)iodo]-*m*-methoxybenzene (11b). To a solution of compound **10a** (2 g, 5.68 mmol) was added to solution of *p*-toluenesulfonic acid monohydrate (1.1 g, 5.7 mmol) in acetonitrile (10 mL) at room temperature under nitrogen atmosphere. The mixture was stirred for 12 h at room temperature. After the addition of diethyl ether and EtOAc (v/v = 2:1, 30 mL), the solid was filtered and dried in vacuo to give the product **11b** as a white solid (92%): mp 134−137 °C (decomp); ^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (s, 3H), 3.52 (s, 3H), 6.81 (d, J = 7.5 Hz, 2H), 6.93−6.95 (m, 1H), 7.17 (d, J = 8.0 Hz, 2H), 7.22 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.52−7.53 (m, 1H), 9.47 (br, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 21.28, 56.53, 114.50, 120.95, 121.95, 126.89, 128.91, 129.84, 133.42, 141.92, 142.94, 162.49.

[(Hydroxy)(tosyloxy)iodo]thiophene (11c). To a solution of compound **10b** (500 mg, 1.52 mmol) was added to solution of *p*-toluenesulfonic acid monohydrate (290 mg, 1.52 mmol) in acetonitrile (10 mL) at room temperature under nitrogen atmosphere. The mixture was stirred for 1 h at room temperature. After the addition of diethyl ether and EtOAc (v/v = 2:1, 30 mL), the solid was filtered and dried in a short time under nitrogen atmosphere to give the product **11c** (95%). Because this product (**11c**) was unstable under air atmosphere and at room temperature, it was used immediately after isolation to prepare the next compound, **12c**: ^{1}H NMR (400 MHz, CD_3OD) δ 2.36 (s, 3H), 4.88 (s, 3H), 7.22 (d, J = 8.0 Hz, 2H), 7.35−7.37 (m, 1H), 7.68 (d, J = 8.4 Hz, 2H), 8.09−8.10 (m, 1H), 8.24−8.25 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 21.30, 126.95, 129.83, 131.19, 141.39, 141.76, 143.29, 143.38, 145.23.

(2*S*)-[2-Benzoylphenylamino]-3-{4-[2-(4-iodophenyl(phenyl) Oxazol 5-methyl-4-yl)ethoxy]phenyl}propionic Acid Methyl Ester Iodonium Tosylate (12a). To a solution of Koser's reagent **11a** (47 mg, 0.12 mmol) in CH_3CN (3 mL) was added tin compound **9** (100 mg, 0.12 mmol) in CH_3CN (3 mL) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 12 h at room temperature under nitrogen atmosphere. The solvent was evaporated using a stream of nitrogen. The crude mixture was dissolved a small amount of EtOH (1.5 mL) and transferred to the centrifuge tube to which was added excess diethyl ether (20 mL). After centrifuging, the collected yellow oil was dried in vacuo to give the product **12a** (42 mg, 38%) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 2.32 (s, 3H), 2.34 (s, 3H), 2.91 (t, J = 6.4 Hz, 2H), 3.06−3.20 (m, 2H), 3.69 (s, 3H), 4.16 (t. J = 6.0 Hz, 2H), 4.56 (t, J = 6.0 Hz, 1H), 6.56−6.60 (m, 1H), 6.73−6.78 (m, 3H), 7.08 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.34−7.71 (m, 12H), 8.02 (d, J = 8.4 Hz, 2H), 8.18−8.24 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 10.15, 21.29, 26.88, 38.36, 52.70, 58.29, 67.26, 113.30, 115.61, 116.13, 116.20, 116.59, 119.37, 126.94, 129.19, 129.70, 129.78, 130.00, 131.49, 132.08, 132.20, 133.24, 133.75, 135.04, 136.25, 136.38, 136.56, 137.03, 141.49, 141.59, 143.56, 148.56, 151.25, 158.68, 159.09, 174.37, 200.81; MS (ESI) *m*/*z* 763 (M^+ − OTs, 100%). HRMS calcd for $C_{41}H_{36}IN_2O_5$ 763.1669, found 763.1657.

(2*S*)-[2-Benzoylphenylamino]-3-{4-[2-(4-iodophenyl(3-methoxyphenyl) oxazol 5-methyl-4-yl)ethoxy]phenyl}propionic Acid Methyl Ester Iodonium Tosylate (12b). Prepared from tin precursor **9** and the *m*-methoxyphenyl iodonium salt **11b** by the method used to prepare iodonium salt **12a** and obtained as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 2.29 (s, 3H), 2.31 (s, 3H), 2.87 (t, J = 6.0 Hz, 2H), 3.03−3.17 (m, 2H), 3.67 (s, 3H), 3.80 (s, 3H), 4.12 (t, J = 6.5 Hz, 2H), 4.53 (t, J = 6.0 Hz, 1H), 6.56 (t, J = 7.0 Hz, 1H), 6.71−6.75 (m, 3H), 7.06 (d, J = 8.5 Hz, 2H), 7.17−7.20 (m, 3H), 7.33−7.49 (m, 8H), 7.66 (d, J = 8.0 Hz, 2H), 7.69−7.71 (m, 1H), 7.79 (t, J = 2.5 Hz, 1H), 7.95 (d, J = 8.5 Hz, 2H), 8.20 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 10.17, 21.33, 26.87, 38.35, 52.75, 56.51, 58.34, 67.27, 113.32, 115.62, 115.95, 116.12, 116.66, 119.39, 119.68, 121.87, 126.94, 126.99, 128.38, 129.25, 129.71, 129.81, 129.85, 130.05, 131.53, 132.12, 132.24, 133.88, 135.07, 136.29, 137.04, 141.55, 141.69, 143.52, 148.62, 151.31, 158.78, 159.15, 162.83, 174.43, 200.90; MS (ESI) *m*/*z* 793 (M^+ − OTs, 100%), 752 (35%). HRMS calcd for $C_{42}H_{38}IN_2O_6$ 793.1775, found 793.1801.

(2*S*)-[2-Benzoylphenylamino]-3-{4-[2-(4-iodophenyl(2-thiophenyl) oxazol 5-methyl-4-yl)ethoxy]phenyl}propionic Acid Methyl Ester Iodonium Tosylate (12c). Prepared from tin precursor **9** and thiophene iodonium salt **11c** by the method used to prepare iodonium salt **12a** and obtained as a yellow oil: ^{1}H NMR (500 MHz, CD_3OD) δ 2.30 (s, 3H), 2.32 (s, 3H), 2.88 (t, J = 6.5 Hz, 2H), 3.04−3.17 (m, 2H), 3.67 (s, 3H), 4.13 (t, J = 6.0 Hz, 2H), 4.54 (t, J = 6.0 Hz, 1H), 6.56 (t, J = 7.5 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 9.0 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H), 7.14−7.16 (m, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.33−7.37 (m, 2H), 7.41−7.44 (m, 2H), 7.48−7.52 (m, 3H), 7.67 (d, J = 8.0 Hz, 2H), 7.86−7.87 (m, 1H), 7.95 (d, J = 9.0 Hz, 2H), 8.01−8.02 (m, 1H), 8.19 (d, J = 8.5

Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 10.13, 21.29, 26.90, 38.39, 52.70, 58.33, 67.28, 99.20, 113.31, 115.63, 116.13, 119.41, 119.55, 126.95, 129.22, 129.70, 129.79, 130.01, 130.93, 131.50, 132.15, 132.22, 135.08, 136.26, 136.38, 136.41, 138.78, 141.56, 141.65, 142.39, 143.52, 148.66, 151.29, 158.73, 159.12, 174.43, 200.92; MS (ESI) *m*/*z* 769 ($M^+ - OTs$, 100%). HRMS calcd for $C_{39}H_{34}IN_2O_5S$ 769.1233, found 769.1238.

Halodestannylation Reactions with Bromine and Iodine. *Synthesis of (2S)-(2-benzoylphenylamino)-3-(4-{2-[2-(4-bromophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (**2**).* To a solution of compound **9** (10 mg, 0.012 mmol) in peracetic acid (500 μL, 0.3% v/v in acetic acid) was added NH_4-Br (1.3 mg, 0.013 mmol) in H_2O (500 μL) at room temperature. The reaction mixture was stirred for 30 min at room temperature, then diluted with 0.5 N NaOH (3 mL) and EtOAc (3 mL). The organic layer was separated and dried with sodium sulfate, and the solvents were removed in vacuo. The crude mixture obtained was dissolved in THF/MeOH (v/v = 3:1, 0.5 mL) and then was treated with 0.1 M LiOH (130 μL, 0.013 mmol). The resulting solution was stirred at room temperature for 1 h. The reaction mixture was then poured into 1 N HCl (20 μL) and extracted with EtOAc. The organic layer was separated and dried with sodium sulfate. The obtained organic layers were concentrated and purified by silica gel flash column chromatography using 5% MeOH/CH_2Cl_2 as eluant to afford the product **2** (7 mg. 91%) as a yellow solid.

*Synthesis of (2S)-(2-benzoylphenylamino)-3-(4-{2-[2-(4-iodophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (**3**).* To a solution of compound **9** (10 mg, 0.012 mmol) in peracetic acid (500 μL, 0.3% v/v in acetic acid) was added NaI (2 mg, 0.013 mmol) in H_2O (500 μL) at room temperature. The reaction mixture was stirred for 30 min at room temperature, then diluted with 0.5 N NaOH (3 mL) and EtOAc (3 mL). The organic layer was separated and dried with sodium sulfate, and the solvents were removed in vacuo. The hydrolysis of methyl ester was performed by the method used for **2** and obtained as a yellow solid (93%).

Fluorination of Iodonium Salts. *Synthesis of (2S)-(2-benzoylphenylamino)-3-(4-{2-[2-(4-fluorophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)propionic Acid (**1**).* To a solution of compound **12c** (10 mg, 0.011 mmol) in CH_3CN (1 mL) was added CsF (2.5 mg, 0.016 mmol). The reaction mixture was heated at 90 °C for 60 min. After cooling, the solvent was removed in vacuo. Hydrolysis of the methyl ester was performed by the method used to prepare compound **2**. The progress of the reaction and yields were analyzed by HPLC (prep silica gel column, 10 μm, 10 × 250 mm; hexane/isopropanol/methylene chloride/trifluorosulfonic acid = 70:1.5:28:0.03 (v/v): 254 nm; 4 mL/min).

PPARγ Binding Affinity Assays. The binding of compound **1** to PPARγ was evaluated using a scintillation proximity assay (SPA) adapted from the method described by Sun et al. (*34*). Briefly, only the energy emitted from the negatrons of the bead-protein-bound radioligand complex can be absorbed by scintillant-coated SPA beads to produce light, which can be detected in a liquid scintillation counter. The potency of the test compound is assessed by its displacement of a tritium-labeled ligand. Yttrium silicate (Ysi) copper His-Tag SPA beads were purchased from Amersham Biosciences (Piscataway, NJ). A histidine-tagged human PPARγ ligand-binding domain (amino acids 195−477) was acquired from Invitrogen (Carlsbad, CA). The tritium ligand, [^{3}H]-rosiglitazone, which has high affinity and high potency for PPARγ (K_d and EC_{50} ca. 15−20 nM) (*35*), was obtained from American Radiolabeled Chemicals (St. Louis, MO).

[^{3}H]-Rosiglitazone and PPARγ protein were diluted to 23 μCi/mL and 50 ng/mL stock, respectively. Radioligand, protein, and test compound at various concentrations (10 nM to 2 μM) were incubated at room temperature for 2 h. SPA beads were diluted and aliquoted into a clear-bottomed 96-well plate according to the manufacturer's specifications. Test ligand, [^{3}H]-Rosiglitazone, and protein were then added to the assay plate and incubated at room temperature for at least 1 h before the plate was read by a Packard TopCount plate reader (Perkin-Elmer, Wellesley, MA). GW1929 (Cayman Chemical Co., Ann Arbor, MI), a potent PPARγ agonist, served as a positive control. Nonspecific binding was very low. The initial IC_{50} values of GW1929 and test compound were calculated by the *Prism* program (GraphPad Software, San Diego, CA). The reported compound IC_{50} value of the compound tested was normalized against GW1929 due to the variability of the assay (*36*).

RESULTS AND DISCUSSION

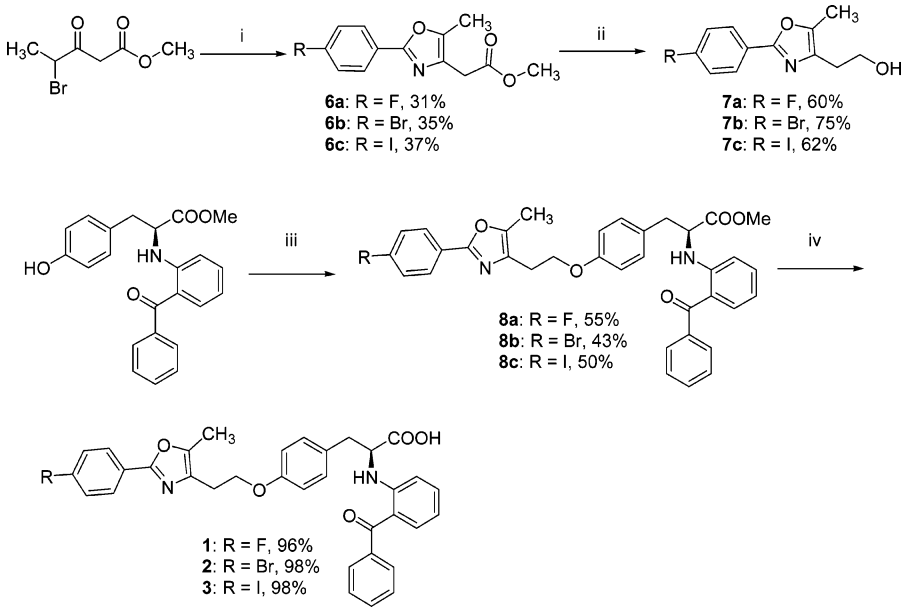
Chemical Synthesis. We prepared compounds **1**−**3** by two approaches: the first (Scheme 2) was to prepare authentic samples of these PPARγ ligands; the second proceeded through precursors from which the halogen could be introduced at a very late step, using a simple halogen reagent (Schemes 3 and 7), as would be needed for labeling these compounds with radiohalogens at high specific activity.

Synthesis of (2*S*)-(2-benzoylphenylamino)-3-(4-{2-[2-(4-halogenophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)-propionic Acid (1−3). As shown in Scheme 2, the three PPARγ ligands (**1**−**3**) were synthesized in four steps, using a modification of known methods (*19*−*21*). Three halogen-substituted phenyl oxazole rings **6a**−**c** were synthesized directly from a 4-halogen-substituted benzamide and 4-bromo-3-oxopentanoate, followed by reduction of the ester with lithium aluminum hydride or sodium borohydride in tetrahydrofuran to produce the corresponding alcohols **7a**−**c**. Using Mitsunobu conditions, alcohols **7a**−**c** were coupled to (2*S*)-2-((2-benzoylphenyl)-amino)-3-(4-hydroxyphenyl)propionic acid methyl ester. Hydrolysis of the methyl esters **8a**−**c** with LiOH solution proceeded under mild conditions to give the desired fluorine, bromine, and iodine-substituted PPARγ ligands **1**−**3**.

Synthesis of (2*S*)-(2-benzoylphenylamino)-3-(4-{2-[2-(4-tributylstannylphenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)-propionic Acid Methyl Ester (9) and Iodonium Tosylates (12a−c). A different approach is needed for the synthesis of the target compounds **1**−**3** in radiolabeled form. Because the radiohalogens have relatively short half-lives (F-18, 110 min; Br-76, 16.1 h; I-124, 4.2 d), it is best if they can be introduced late in the synthesis, in a rapid and efficient manner, into appropriate precursors using simple halogenating agents that are available in radiolabeled form at high specific activity. The trialkylstannane compound **9**, which can readily be prepared from the iodo compound **8c** in the presence of bis(tributyl)tin and a catalytic quantity of tetrakis(triphenylphosphine)palladium (Scheme 3), is a precursor that could be used to prepare the bromine- and iodine-substituted PPARγ ligands **2** and **3** in labeled form by a halodestannylation reaction. These reactions proceed readily (Scheme 3).

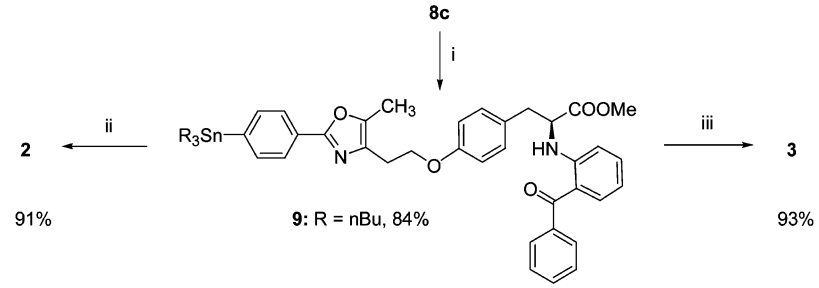
We considered a number of approaches that would be suitable for the preparation of the fluorine-substituted PPARγ ligand (**1**) in F-18 labeled form at the high specific activity required for PET imaging of nuclear receptors. For this type of tracer-level labeling, [^{18}F]fluoride ion is the only practical fluorination reagent. Fluoride ion can be introduced into certain aromatic rings by nucleophilic aromatic substitution of a suitable leaving group, such as nitro or trimethylammonium triflate, provided the ring is sufficiently activated by ortho or para electron withdrawing groups (*37*−*39*). In our system, it was not clear whether the 2-oxazolyl substituent at the para position would provide sufficient activation for fluoride ion substitution.

Scheme 2 [a]



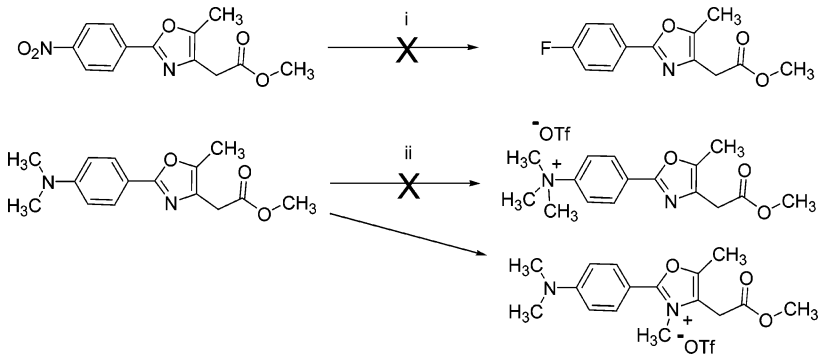
[a] Reaction conditions: (i) 4-halobenzamide, 1,2-dichlorobenzene, 130 °C, 24 h; (ii) LAH, THF, 0 °C, 1 h, or $NaBH_4$, MeOH, rt, 6 h; (iii) **7a**−**c**, PPh_3, DEAD, THF, 0 °C ∼ rt, 12 h; (iv) LiOH, THF/MeOH, rt, 6 h.

Scheme 3 [a]



[a] Reaction conditions: (i) $(PPh_3)_4Pd$, Sn_2Bu_6, toluene, 120 °C, 12 h; (ii) (a) NH_4Br, CH_3CO_3H, CH_3COOH, rt, 30 min, (b) LiOH, THF/MeOH, rt, 1 h; (iii) (a) NaI, CH_3CO_3H, CH_3COOH, rt, 30 min, (b) LiOH, THF/MeOH, rt, 1 h.

Scheme 4 [a]

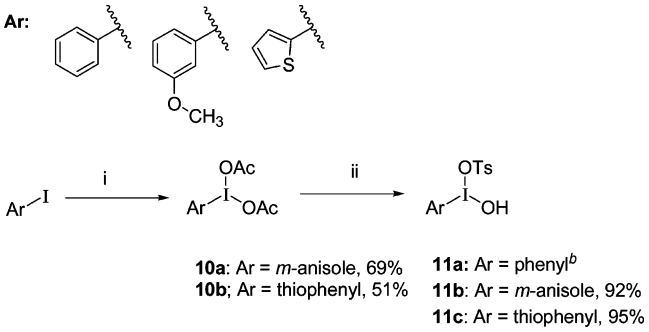


[a] Reaction conditions: (i) TMAF, DMSO, 130 °C; (ii) $CF_3SO_2OCH_3$, CH_2Cl_2, rt.

As shown in Scheme 4, in studies on a model system, we were unable to effect fluoride ion substitution on the corresponding p-nitro precursor under a variety of conditions. Curiously, we were also unable to prepare the more reactive p-trimethylammonium precursor, because in the final methylation step of the dimethylaniline precursor, the oxazole moiety became N-methylated in preference to the aromatic amine, giving the N-methyloxazolium salt as the only identifiable product. Although alternative routes to reach this precursor were considered, the uncertainty of success with this modestly activated ring system led us to consider different approaches altogether.

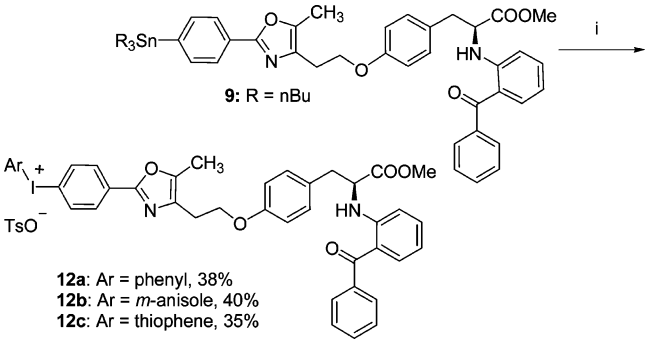
It was known that diaryliodonium salts can be used as precursors for fluoroarenes (*27*), and Widdowson showed that these salts are useful, as well, for the introduction of fluorine-18 into arenes (*28*). Mixed functional diaryliodonium tosylates can be prepared by the reaction of aryltrialkylstannanes with hydroxy(tosyloxy)iodobenzene (Koser's reagent, **11a**) under mild conditions (*26*−*29*). To prepare diaryliodonium precursors suitable for introducing fluorine into the PPARγ ligand **1** (Scheme 5), we reacted the tributyltin analog **9** with commercially available Koser's reagent, as well as with two other related and known hydroxy(tosyloxy)iodoarenes (**11b** and **11c**) derived, respectively, from *m*-methoxyphenyl iodide (*m*-io-

Scheme 5 [a]



[a] Reaction conditions: (i) $NaIO_4$, AcONa, AcOH, Ac_2O, 120 °C, 2 h; (ii) TsOH, CH_3CN, rt, 2 h. [b] Koser's reagent **10a** was purchased from Aldrich.

Scheme 6 [a]



[a] Reaction conditions: (i) **11a**−**c**, CH_2Cl_2, rt, 12 h.

doanisole) and 2-iodothiophene (*31*−*33*). These reactions proceeded readily and in good yield under mild conditions.

The diaryliodonium salts were readily prepared from the same tin precursor **9** that was used to prepare the bromo and iodoanalogs (**2** and **3**) by exposure to the three hydroxyl(tosyloxy)iodoarenes **11a**−**c** (Scheme 6). These iodonium salts are liquids that can be easily isolated, but they cannot be rigorous purified. They are sufficiently pure, however, to function as effective precursors for fluorination to produce the final compound **1** (Scheme 7).

In fluorination of mixed functional diaryliodonium salts, steric demand and/or electron deficiency of the aromatic rings determines the site of fluorine substitution (*27*, *28*, *40*, *41*). Because steric effects in the two rings of our systems are essentially equivalent, fluoride anion is expected to attack the more electron deficient ring. Thus, the three salts we have prepared present an interesting situation, where the "dispensable ring" is either phenyl or the two more electron rich rings, *m*-methoxyphenyl or 2-thiophenyl. We avoided the choice of the *p*-methoxyphenyl analog of compound **11b** series, because the reagent required, [hydroxy(tosyloxy)iodo]-*para*-methoxybenzene, is known to be unstable and to decompose violently at room temperature (*42*). The reagent to prepare the thiophene heteroaromatic iodonium salt **12c**, namely, [hydroxy(tosyloxy)-iodo]thiophene **11c**, is also somewhat unstable; however, we were able to isolate it and use it immediately to prepare the PPARγ precursor **12c** (*33*).

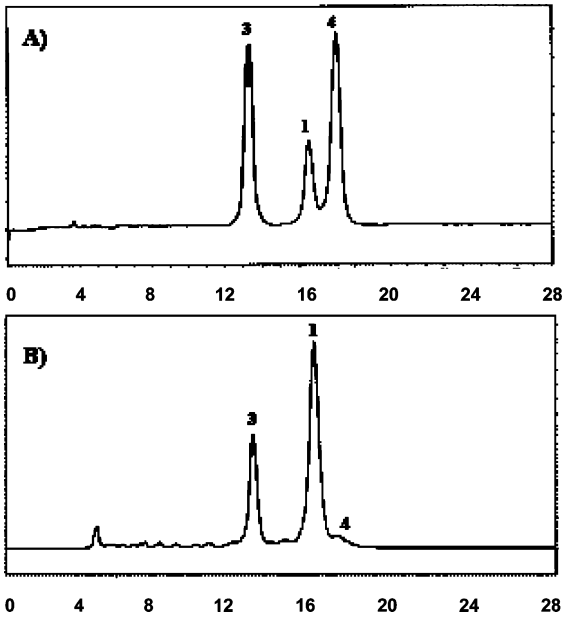


Figure 1. (A) HPLC profile of the standards. t_R = 16.7 min (**1**), 13.4 min (**3**), and 18.0 min (**4**). (B) HPLC analysis of the reaction mixture after fluorination of **12c**, followed hydrolysis.

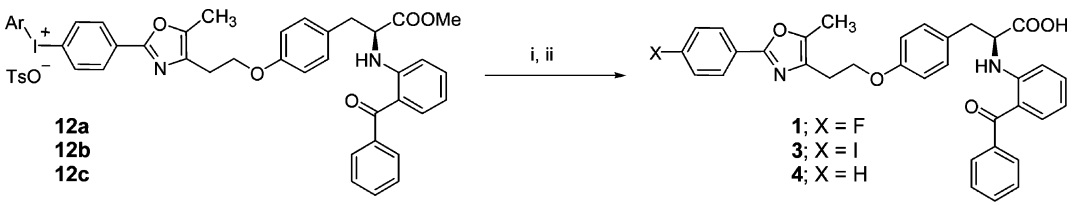
Table 1. Reaction of Fluorination with Iodonium Tosylates

		product ratio (%)[a]			
cmpd no	Ar	**1**	**3**	**4**	yields[b]
12a	phenyl	50	38	9	27%
12b	*m*-anisolyl	34	56	5	28%
12c	2-thiophenyl	68	28	1	35%

[a] Progress of the reaction and yields were analyzed by HPLC (prep. silica gel column, 10 μ, 10 × 250 mm; hexane/isopropanol/methylene chloride/trifluorosulfonic acid = 70:1.5:28.5:0.03 (v/v); 254 nm; 4 mL/min). Ratios represent the areas of the peaks associated with products **1**, **3**, and **4**; the ratios do not add to 100% because small byproduct peaks are also present. [b] Yield of isolated pure product **1**, obtained by preparative column chromatography.

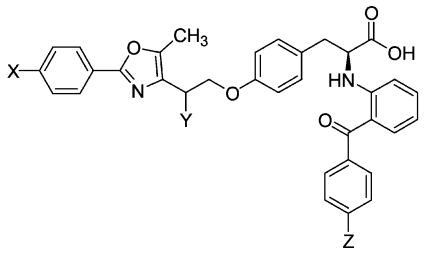
Synthesis of (2*S*)-(2-Benzoylphenylamino)-3-(4-{2-[2-(4-fluorophenyl)-5-methyloxazol-4-yl]ethoxy}phenyl)-propionic Acid (1). Various conditions were explored for the preparation of the fluoride-substituted PPARγ ligand **1** from the three precursors **12a**−**c** (Scheme 7). The results from reactions conditions optimized to give the fluorine-substituted ester (CsF in acetonitrile at 90 °C) and then mild hydrolysis to the acid with LiOH are summarized in Table 1. Reaction products were analyzed by HPLC, and the crude reaction mixture showed the presence of the desired product **1** (t_R = 16.7 min) and two side products (t_R = 13.4 and 18.0 min, Figure 1). These were identified as the iodine-substituted and reduced compounds (**3** and **4**, respectively) by comparison with authentic samples that we had prepared (cf., Scheme 2). Product ratios of **1**, **3**, and **4** were calculated from the peak areas, and the

Scheme 7 [a]



[a] Reaction conditions: (i) CsF, CH_3CN, 90 °C, 50 min; (ii) 0.5 M LiOH 80 °C, 5 min.

Table 2. Three New Fluorine-Substituted Ligands for PPARγ Receptors and Their Binding Affinities[a]



	X	Y	Z	
1	F	H	H	
2	Br	H	H	
3	I	H	H	
4	H	H	H	[farglitazar]
5	--	--	--	[see Scheme 1]
12	H	F	H	
13	H	H	F	

	pK_i [b] (nM)		
cmpd no	PPARγ	PPARα	PPARδ
1[c]	9.46 (9.42 ± 0.02)[a]	7.28	6.27
2[a]	9.61 ± 0.03	ND	ND
3[a]	9.70 ± 0.01	ND	ND
4[d]	8.93	6.31	ND
5[e]	8.15	7.60	5.55
12[c]	8.51	5.84	<5.52
13[c]	8.13 (9.85 ± 0.01)[a]	6.04	<5.52

[a] Binding affinities were determined in this study by a competitive radiometric binding assays using scintillation proximity methods; see Experimental Section. ND = not determined. [b] pK_i = −log of the concentration of test compound required to achieve an apparent K_i value. [c] K. C. Lee, unpublished.[d] ref 20. [e] ref 17.

isolated yield of compound **1** was determined after purification by preparative chromatography.

The desired product, compound **1**, was produced in highest product ratio and highest yield from the 2-thiophenyl iodonium salt (**12c**). This is consistent with the fact that, in this salt, the dispensable ring is a thiophene, the most electropositive ring of the three, and thus it is most effective in directing fluoride ion to attack the other ring, that of the complex aryl system of the PPARγ portion of the salt, as is needed to produce the desired product (**1**). The isolated yields of **1** produced from the other salts, **12a** and **12b** were only somewhat lower than that from **12c**. Notably, the unsubstituted product, **4**, potentially an undesired competitor for binding to PPARγ, is also produced in lowest amounts from the thiophenyl precursor **12c**.

As a matter of practical importance, it is also of note that in this chromatographic system the desired fluorine-substituted compound **1** elutes well behind the iodine-substituted byproduct (**3**), and it also precedes the unsubstituted compound (**4**, farglitazar itself), which in any case is produced in very small amounts. Thus, it should be possible to separate the fluorine-18 labeled product from the two reaction byproducts that have potential for competing for its binding to PPARγ, thus ensuring high effective specific activity.

Binding Affinity Determination. In previous work, we synthesized fluorine-substituted members of the benzophenone−tyrosine class (farglitazar) and demonstrated that they had very high binding affinity and selectivity for PPARγ receptor. The result of PPARγ binding measurements on compounds **1**−**4**, done using a scintillation proximity assay (*34*), and measurements of certain related ligands from other studies are shown in Table 2 (*17*, *20*, Lee, K. C., unpublished results). Fluorine substitution of the phenyl ring or alkyl chain of the 2-phenyloxazole system (compounds **1** and **13**) and fluorine substitution of benzophenone ring (compound **12)** provided higher-affinity and more selective PPARγ ligands (Lee, K. C., unpublished results) than did the fluorine-substituted analog of SB 213068 (**5**) that we prepared earlier (*17*). Among these, compound **1** appears to be an excellent ligand in terms of binding affinity and selectivity, and the pK_i value we obtained in this study is very close to that determined on this compound by a corporate laboratory (Lee, K. C., unpublished results).

In this study, we have also evaluated the effect of substitution of this phenyl ring with other halogen isotopes that could be used for PET or SPECT imaging, and both the bromine and iodine analogs (**2** and **3**) retain very high affinity for PPARγ, comparable to or greater than that of the fluorine-substituted compound **1** and the parent ligand farglitazar **4** (Table 2).

CONCLUSION

PPARγ, a nuclear receptor that plays critical roles in regulating lipid and carbohydrate metabolism and cellular differentiation and has marked effects on certain cancers, is an important target for in vivo imaging with PET. In our effort to develop radiohalogen-labeled ligands suitable for this purpose, we have prepared three homologous halogen-substituted analogues (fluorine, bromine, and iodine) of the selective and high-affinity PPARγ ligand **4** (farglitazar). All members of this benzophenone−tyrosine class retain very high affinity for PPARγ, superior to that of F-18 labeled ligands of the phenylpropanoic acid class that we investigated earlier (*17*). We have also developed methods by which the halogen can be introduced into each of these ligands (**1**−**3**) as the penultimate step: the heavy halogens are introduced by an electrophilic destannylation sequence and the fluorine by nucleophilic substitution on an optimized heteroarylarene iodonium salt. The latter reaction is an example of fluorine substitution on one of the most complex, highly functionalized systems using this approach. These strategies are easily adaptable to radiolabeling with these halogens in radiolabeled form. Further work describing the preparation of these compounds in radiolabeled form and evaluation of their in vivo tissue distribution will be described in forthcoming publications.

ACKNOWLEDGMENT

We are grateful for support of this work by grants from the Department of Energy (FG02 86ER60401 to J.A.K.) and the National Institute of Health (P01 HL13851 to M. J. Welch, P.I.).

LITERATURE CITED

(1) Issemann, I., and Green, S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature (London) 347*, 645−650.

(2) Greene, M. E., Blumberg, B., McBride, O. W., Yi, H. F., Kronquist, K., Kwan, K., Hsieh, L., Greene, G., and Nimer, S. D. (1995) Isolation of the human peroxisome proliferator activated receptor gamma cDNA: Expression in hematopoietic cells and chromosomal mapping. *Gene Express. 4*, 281−289.

(3) Schmidt, A., Endo, N., Rutledge, S. J., Vogel, R., Shinar, D., and Rodan, G. A. (1992) Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol. Endocrinol. 6*, 1634−1641.
(4) Lemberger, T., Desvergne, B., and Wahli, W. (1996) Peroxisome proliferator-activated receptors: A nuclear recepor signaling pathway in lipid physiology. *Annu. Rev. Cell Dev. Biol. 12*, 335−363.
(5) Willson, T. M., Cobb, J. E., Cowan, D. J., Wiethe, R. W., Correa, I. D., Prakash, S. R., Beck, K. D., Moore, L. B., Kliewer, S. A., and Lehmann, J. M. (1996) The structure−activity relationship between peroxisom proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. *J. Med. Chem. 39*, 665−668.
(6) Brun, R. P.;, Kim, J. B., Hu, E., and Spiegelman, B. M. (1997) Peroxisome proliferator-activated receptor gamma and the control of adipogenesis. *Curr. Opin. Lipidol. 8*, 212−218.
(7) Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I., Morris, D. C., and Lehmann, J. M. (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differntiation. *Cell 83*, 813−819.
(8) Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., Mangelsdorf, D. J., Umesono, K., and Evans, R. M. (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. U.S.A. 91*, 7355−7359.
(9) Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) mPPAR γ2: Tissue-specific regulator of an adipocyte enhancer. *Genes Dev. 8*, 1224−1234.
(10) Mueller, E., Smith, M., Sarraf, P., Kroll, T., Aiyer, A., Kaufman, D. S., Oh, W., Demetri, G., Figg, W. D., Zhou, X. P., Eng, C., Spiegelman, B. M., and Kantoff, P. W. (2000) Effects of ligand activation of peroxisome proliferator-activated receptor gamma in human prostate cancer. *Proc. Natl. Acad. Sci. U.S.A. 97*, 10990−10995.
(11) Muller, E., Sarraf, P., Tontonoz, P., Evans, R. M., Martin, K. J., Zhang, M., Fletcher, C. D., Singer, S., and Spiegelman, B. M. (1998) Terminal differentiation of human breast cancer through PPAR gamma. *Mol. Cell 1*, 465−470.
(12) Demetri, G. D., Fletcher, C. D. M., Mueller, E., Sarraf, F., Naujoks, R., Campbell, N., Spiegelman, B. M., and Singer, S. (1999) Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-γ ligand troglitazone in patients with liposarcoma. *Proc. Natl. Acad. Sci. U.S.A. 96*, 3951−3956.
(13) Tontonoz, P., Singer, S., Froman, B. M., Sarraf, P., Fletcher, J. A., Fletcher, C. D. M., Brun, R. P., Mueller, E., Altiok, S., Oppenheim, H., Evans, R. M., and Spiegelman, B. M. (1997) Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. *Proc. Natl. Acad. Sci. U.S.A. 94*, 237−241.
(14) Burstein, H. J., Demetri, G. D., Muller, E., Sarraf, P., Spiegelman, B. M., and Winer, E. P. (2003) Use of the peroxisome proliferator-activated receptor (PPAR) γ ligand troglitazone as treatment for refractory breast cancer: a phase II study. *Breast Cancer Res. Treat. 79*, 391−397.
(15) Wang, Xin., Southard, R. C., and Kilgore, M. W. (2004) The increased expression of peroxisome proliferator-activatated receptor-γ1 in human breast cancer is mediated by selective promoter usage. *Cancer Res. 64*, 5592−5596.
(16) Berger, J P., Akiyama, T. E., and Meinke, P. T. (2005) PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol. Sci. 26*, 244−251.
(17) Kim, S-H., Jonson, S. D., Welch, M. J., and Katzenellenbogen, J. A. (2001) Fluorine-Substituted ligands for the peroxisome proliferator-activated receptor gamma (PPARγ): Potential imaging agents for metastatic tumors. *Bioconjugate Chem. 12*, 439−450.
(18) Gray, S. L., Dalla Nora, E., and Vidal-Puig, A. J. (2005) Mouse models of PPAR-gamma deficiency: dissecting PPAR-gamma's role in metabolic homoeostasis. *Biochem. Soc. Trans. 33*, 1053−1058.
(19) Henke, B. R., Blanchard, S. G., Brackeen, M. F., Brown, K. K., Cobb, J. E., Collins, J. L., Harrington, W. W., Jr., Hashim, M. A., Hull-Ryde, E. A., Kaldor, I., Kliewer, S. A., Lake, D. H., Leesnitzer, L. M., Lehmann, J. M., Lenhard, J. M., Orband-Miller, L. A., Miller, J. F., Mook, R. A., Jr., Noble, S. A., Oliver, W., Jr., Parks, D. J., Plunket, K. D., Szewczyk, J. R., and Willson, T. M. (1998) *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. *J. Med. Chem. 41*, 5020−5036.
(20) Collins, J. L., Blanchard, S. G., Boswell, E. G., Charifson, P. S., Cobb, J. E., Henke, B. R., Hull-Ryde, E. A., Kazmierski, W. M., Lake, D. H., Leesnitzer, L. M., Lehmann, J., Lenhard, J. M., Orband-Miller, L. A., Gray-Nunez, Y., Parks, D. J., Plunkett, K. D., and Tong, W. Q. (1998) *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 2. Structure−activity relationship and opitimization of the phenyl alkyl ether moiety. *J. Med. Chem. 41*, 5037−5054.
(21) Cobb, J. E., Blanchard, S. G., Boswell, E. G., Brown, K. K., Charifson, P. S., Cooper, J. P., Collins, J. L., Dezube, M., Henke, B. R., Hull-Ryde, E. A., Lake, D. H., Lenhard, J. M., Oliver, W., Jr., Oplinger, J., Pentti, M., Parks, D. J., Plunket, K. D., and Tong, W. Q. (1998) *N*-(2-Benzoylphenyl)-L-tyrosine PPARγ agonists. 3. Structure−activity relationship and opitimization of the N-aryl substituent. *J. Med. Chem. 41*, 5055−5069.
(22) Willson, T. M., Brown, P. J., Sternbach, D. D., and Henke, B. R. (2000) The PPARs: From orphan receptors to drug discovery. *J. Med. Chem 43*, 527−550.
(23) Haigh, D., Allen, G., Birrell, H. C., Buckle, D. R., Cantello, B. C., Eggleston, D. S., Haltiwanger, R. C., Holder, J. C., Lister, C. A., Pinto, I. L., Rami, H. K.;, Sime, J. T., Smith, S. A., and Sweeney, J. D. (1999) Nonthiazolidinedione antihyperglycaemic agents. Part 3: The effects of stereochemistry on the potency of alpha-methoxy-beta-phenylpropanoic acids. *Bioorg. Med. Chem. 7*, 821−830.
(24) Young, P. W., Buckle, D. R., Cantello, B. C., Chapman, H., Clapham, J. C., Coyle, P. J., Haigh, D., Hindley, R. M., Holder, J. C., Kallender, H., Latter, A. J., Lawrie, K. W., Mossakowska, D., Murphy, G. J., Roxbee, C. L., and Smith, S. A. (1998) Identification of high-affinity binding sites for the insulin sensitizer resiflitazone (BRL-49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor gamma. *J. Pharmacol. Exp. Ther. 284*, 751−759.
(25) Katzenellenbogen, J. A., Heiman, D. F., Carlson, K. E., and Lloyd, J. E. (1982) *In vivo* and *in vitro* steroid receptor assays in the design of estrogen pharmaceuticals. In *Receptor Binding Radiotracers* (Eckelman, W. C., Ed.), Vol. 1, Chapter 6, pp 93−126, CRC Press, Boca Raton.
(26) Koser, G. F., Wettach, R. H., and Smith, C. S. (1980) New methodology in iodonium salt synthesis. Reaction of [hydoxy-(tosyloxy)iodo]arenas with aryltrimethylsilanes. *J. Org. Chem. 45*, 1542−1543.
(27) van der Puy, M. (1982) Conversion of diaryliodonium salts to aryl fluorides. *J. Fluorine Chem. 21*, 385−392.
(28) Shah, A., Pike, V. W., and Widdowson, D. A. (1998) The synthesis of [^{18}F]fluoroarenes from the reaction of cyclotron-produced [^{18}F]-fluoride ion with diaryliodonium salts. *J. Chem. Soc., Perkin Trans. 1*, 2043−2046.
(29) Pike, V. W., Butt, F., Shah, A., and Widdowson, D. A. (1999) Facile synthesis of substituted diaryliodonium tosylates by treatment of aryltributylstannanes with Koser's reagent. *J. Chem. Soc., Perkin Trans. 1*, 245−248.
(30) Pangborn, A. B., Giardello, M. A., Grubbs, R. H., Rosen, R. K., and Timmer, F. J. (1996) Safe and convenient procedure for solvent purification. *Organometallics 15*, 1518−1520.
(31) Kazmierczak, P., Skulski, L., and Kraszkiewicz, L. (2001) Synthesis of (diacetoxyiodo)arenas of iodylarenes from iodoarenes, with sodium periodate as the oxidant. *Molecules 9*, 881−891.
(32) Togo, H., Nabana, T., and Yamaguchi, K. (2000) Preparation and reactivities of novel (diacetoxyiodo)arenas bearing heteroaromatics. *J. Org. Chem. 65*, 8391−8394.
(33) Nabana, T., and Togo, H. (2002) Reactivities of novel [hydoxy-(tosyloxy)iodo]arenas and [hydoxy(phosphoryloxy)iodo]arenas for α-tosyloxylation and α-phosphoryloxylation of ketones. *J. Org. Chem. 67*, 4362−4365.
(34) Sun, S., Almaden, J., Carlson, T. J., Barker, J., and Gehring, M. R. (2005) Assay development and data analysis of receptor-ligand binding based on scintillation proximity assay. *Metab. Eng. 7*, 38−44.
(35) Xu, H. E., Lambert, M. H., Montana, V. G., Plunket, K. D., Moore, L. B., Collins, J. L., Oplinger, J. A., Kliewer, S. A., Gampe, R. T., Jr., McKee, D. D., Moore, J. T., and Willson, T. M. (2001) Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. U.S.A. 98*, 13919−13924.

(36) Brown, K. K., Henke, B. R., Blanchard, S. G., Cobb, J. E., Mook, R., Kaldor, I., Kliewer, S. A., Lehmann, J. M., Lenhard, J. M., Harrington, W. W., Novak, P. J., Faison, W., Binz, J. G., Hashim, M. A., Oliver, W. O., Brown, H. R., Parks, D. J., Plunket, K. D., Tong, W. Q., Menius, J. A., Adkison, K., Noble, S. A., and Wilson, T. M. (1999) A novel N-aryl tyrosine activator of peroxisome proliferator-activated receptor-gamma reverses the diabetic phenotype of the Zucker diabetic fatty rat. *Diabetes 48*, 1415−1424.

(37) Stone-Elander, S., and Elander, N. (1993) Fast chemistry in microwave fields: Nucleophilic ^{18}F-radiofluorinations of aromatic molecules. *Appl. Radiat. Isot. 44*, 889−893.

(38) Ludwig, T., Ermert, J., and Coenen, H. H. (2002) 4-[^{18}F]fluoroaryl alkyl ethers via an improved synthesis of n.c.a. 4-[^{18}F]fluorophenol. *Nucl. Med. Biol. 29*, 255−262.

(39) Haka, M. S., Kilbourn, M. R., Watkins, G. L., and Toorongian, S. A. (1989) Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [^{18}F]fluorides: improved synthesis of [^{18}F]GBR-13119. *J. Labelled Compd. Radiopharm. 27*, 823−833.

(40) Grushin, V. V. (1992) Carboranylhalonium ions: From striking reactivity to a unified mechanistic analysis of polar reactions of diarylhalonium compounds. *Acc. Chem. Res. 25*, 529−536.

(41) Grushin, V. V., Demkina, I. I., and Tolstaya, T. P. (1992) Unified mechanistic analysis of polar reactions of diaryliodonium salts. *J. Chem. Soc., Perkin Trans. 2*, 505−511.

(42) Papoutsis, I., Spyroudis, S., Varvoglis, A., and Raptopoulou, C. P. (1997) Arylidonium derivatives of 2-amino-1,4-quinones: Preparation and reactivity. *Tetrahedron 53*, 6097−6112.

BC060191G