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# One-pot synthesis and biodistribution of fluorine-18 labeled serum albumin for vascular imaging

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ABSTRACT

*Introduction:* Equilibrium single-photon radionuclide imaging methods for assessing cardiac function and the integrity of the vascular system have long been in use for both clinical and research purposes. However, positron-emitting blood pool agents that could provide PET equivalents to these (and other) clinical procedures have not yet been adopted despite technical imaging advantages offered by PET. Our goal was to develop a PET blood pool tracer that not only meets necessary in vivo biological requirements but can be produced with an uncomplicated and rapid synthesis method which would facilitate clinical translation. Herein, albumin labeled with fluorine-18 was synthesized using a one-pot method and evaluated in vitro and in vivo in rats.

*Methods:* A ligand (NODA-Bz-TFPE), containing NODA attached to a tetrafluorophenylester (TFPE) via a phenyl linker (Bz), was labeled with aluminum fluoride (Al[$^{18}$F]F). Conjugation of the serum albumin with the ligand (Al[$^{18}$F]F-NODA-Bz-TFPE), followed by purification (size exclusion chromatography), yielded the final product (Al[$^{18}$F]F-NODA-Bz-RSA/HSA). In vitro stability was evaluated in human serum albumin by HPLC. Rat biodistributions and whole-body PET imaging over a 4 h time course were used for the in vivo evaluation.

*Results:* This synthesis exhibited an overall radiochemical yield of 45 ± 10% (n = 30), a 50-min radiolabeling time, a radiochemical purity >99% and apparent stability up to 4 h in human serum. Blood had the highest retention of Al[$^{18}$F]F-NODA-Bz-RSA at all times with a blood half-life of 5.2 h in rats. Al[$^{18}$F]F-NODA-Bz-RSA distribution in most rat tissues remained relatively constant for up to 1 h, indicating that the tissue radioactivity content represents the respective tissue plasma volume. Dynamic whole-body PET images were in agreement with these findings.

*Conclusions:* A new ligand has been developed and radiolabeled with Al[$^{18}$F]F that allows rapid (50-min) preparation of fluorine-18 serum albumin in one-pot. In addition to increased synthetic efficiency, the construct appears to be metabolically stable in rats. This method could encourage wider use of PET to quantify cardiac function and tissue vascular integrity in both research and clinical settings.



## 1. Introduction

Radionuclide procedures based on imaging the equilibrium distribution of a radiotracer distributed uniformly throughout the blood pool have proven extremely useful in both clinical and research settings [1–6]. Depending on the specific properties of the agent, procedures have been devised that include locating sites of regional hemorrhage [7, 8], assessing vascular permeability in cancerous tissues [9], identifying sites of tissue leakage and inflammation in response to traumatic injury [10] and, commonly, visualization and quantification of cardiac left ventricular function in patients with heart disease [11, 12] and those undergoing cancer chemotherapy with drugs that compromise cardiac function [13, 14].

Today, most of these imaging procedures are carried out with gamma camera-based SPECT imaging systems and single-photon labeled tracers, e.g. $^{99m}$Tc-labeled red blood cells. In contrast, routine clinical blood pool imaging with PET is uncommon which is most likely a consequence of the lack of a comparable, clinically approved PET agent. Nonetheless, equilibrium PET imaging of the vascular system offers several compelling advantages over single photon methods, particularly if the radiolabel is fluorine-18 (because of its nuclear and chemical properties). It is not surprising, therefore, that a variety of fluorine-18 labeled prosthetic groups have been used to radiolabel human (HSA) and rat (RSA) serum albumin, e.g. 2,3,5,6-tetrafluorophenyl penta-[$^{18}$F]fluorobenzoate [15], 4-nitrophenyl-2-[$^{18}$F]fluoropropionate [16], methyl 3-[$^{18}$F]fluoro-5-nitrobenzimidate [17], 4-[$^{18}$F]fluorophenacylbromide [17], 4-azidophenacyl-[$^{18}$F]fluoride ([$^{18}$F]APF) [16], [$^{18}$F]fluorobenzaldehyde ([$^{18}$F]FBA) [18], [$^{18}$F]SFB [16],

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$^{18}$F-labeled sulfonamide-based click chemistry building blocks [19] and a Si[$^{18}$F]F bonded prosthetic groups [20, 21]. However, the radiolabeled products resulting from these methods exhibit differences in in vivo biodistribution properties that most likely result from conditions prevailing during synthesis or alterations in metabolism due to differences between prosthetic groups and their linkage to the protein [9, 21, 22].

We have previously reported the synthesis of fluorine-18 labeled albumin ([$^{18}$F]F-NE-HSA/RSA, Fig. 1) [23] in a 90-min synthesis, using Olberg's ligand [$^{18}$F]fluoronicotinate ([$^{18}$F]F-NE) [24], that has favorable biodistribution properties. This agent remains in the blood pool for an extended period of time compared to other serum proteins (i.e. transferrin) and accumulates slowly in the tissues of the major body organ (with the exception of the kidneys). As a result, this compound can be used in applications that include measurement of physical specific organ plasma volume (the physical plasma volume of blood in the organ per unit mass of organ tissue), visualizaton and quantification of cardiac function by ECG-gated imaging of the heart, and measurement of specific organ plasma distribution volumes to assess vascular "leakage" in cancerous and inflamed tissues. Importantly, this radiolabeled albumin exhibited stable tissue distributions over 4 h indicating that this agent could be used to make multiple tissue plasma volume measurements over time periods several hours long to assess the effects of interventions, an experimentally useful paradigm.

We sought to investigate an alternative procedure to radiolabel serum albumin (HSA/RSA) using an Al[$^{18}$F]F chelate, with the intention of shortening and simplifying the synthetic process while retaining the advantageous properties of the [$^{18}$F]F-NE-RSA/HSA compound. McBride et al. were the first to demonstrate the use of a one step AlF method to radiolabel biomolecules with high molar activity [25]. Among all the ligands tested, the pentadentate ligand, 1,4,7- triazacyclononane-1,4-diacetic acid (NODA) [26–28] was the preferred ligand for the Al[$^{18}$F]F method. In this labeling method, chelation of Al[$^{18}$F]F with NODA was achieved at elevated temperature (100 °C) in one step. This condition is not suitable for proteins since most proteins are unstable at this temperature. McBride et al. have also reported a two-step indirect method for labeling of temperature sensitive biomolecules [28] based on labeled maleimide conjugation with a thiol functionality of protein. Other groups have subsequently adopted this method (one step/two-step) to radiolabel a variety of biomolecules [29–33]. In this work, we report a new ligand (NODA-Bz-TFPE) for conjugation with amine functionalities of protein to synthesize fluorine-18 labeled albumin and compare the bio-distribution properties of this agent to those observed in our previous work [23] and to other literature compounds [18, 21]. This method was based on the synthesis of a NODA ligand functionalized with tetrafluorophenyl ester (NODA-Bz-TFPE) followed by labeling of the ligand with Al[$^{18}$F]F. The labeled ligand was then conjugated to the amine functionalities of albumin and purified by size exclusion chromatography using a mini-Trap column to produce the final labeled albumin product which was then evaluated in vitro and in vivo.

## 2. Materials and methods

Di-tert-butyl 2,2′-(1,4,7-triazonane-1,4-diyl)diacetate was purchased from CheMatech, France (Dijon, France) and used as received.

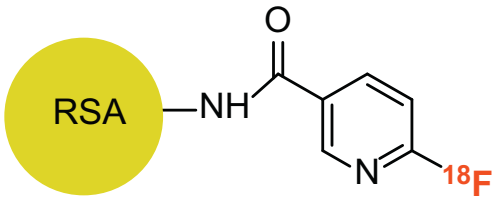


**Fig. 1.** Structure of [$^{18}$F]F-NE-RSA.

Whole human serum was obtained from MP Biomedicals, LLC (Solon, OH, USA). Fluorine-18 was received from National Institutes of Health cyclotron facility (Bethesda, MD, USA). Phosphate-buffered saline (PBS) 1X (12 mM phosphate buffer, pH 7.4, 137 mM NaCl, and 2.7 mM KCl) was obtained from Life Technologies (Carlsbad, CA, USA). All other chemicals and solvents were received from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. PD10 MiniTrap™ columns were obtained from GE Healthcare Bioscience (Pittsburg, PA, USA). Chromafix 30-PS-$HCO_3$ anion-exchange cartridge was purchased from Macherey-Nagel (Düren, Germany) and used as received. Other columns, the Sep-Pak® cartridges and the iTLC-SG plates used in this synthesis were obtained from Agilent Technologies (Santa Clara, CA, USA) and Waters (Milford, MA, USA), respectively. Mass spectrometry (MS) was performed on a 6130 Quadrupole LC/MS, Agilent Technologies instrument equipped with a diode array detector. $^{1}$H, $^{13}$C and $^{19}$F NMR spectra were recorded on a Varian spectrometer (400 MHz). Chemical shifts (ppm) are reported relative to the solvent residual peaks of acetonitrile ($\delta$ $^{1}$H, 2.50 ppm; $^{13}$C 118.26, 1.79). $^{19}$F NMR spectra are reported with reference to the trifluoracetic acid ($\delta$ $^{19}$F, −76.72 ppm). High-resolution mass spectra were recorded on an ESI-TOF. HPLC purification and analytical HPLC analysis were performed on an Agilent 1200 Series instrument equipped with multi-wavelength detectors along with a flow count radiodetector (Eckert & Ziegler, B-FC-3500 diode). iTLC-SG papers were developed using a mixture of acetonitrile and water (3:1, v/v). The papers were read in an Eckert & Ziegler TLC scanner (B-AR2000-1).

HPLC conditions: Method A; Column: Agilent XDB C18 column (9.4 × 250 mm, 5 μm). Mobile phase: A: water (0.1% TFA); B: acetonitrile (0.1% TFA). Gradient: 40–57% B in 7-min; flow rate of 4 mL/min. Method B; Column: Agilent XDB C18 column (4.6 × 150 mm, 5 μm). Mobile phase: A: water (0.1% TFA); B: acetonitrile (0.1% TFA). Gradient: 5–70% B in 15-min; flow rate of 1 mL/min. Method C; Column: Agilent Eclipse GF 250, 5 μm, 4.6 × 250 mm column, PBS buffer (pH 7.4), flow rate: 1 mL/min.

### 2.1. Synthesis of ligand

#### 2.1.1. Synthesis of 4-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)methyl)benzoic acid (Scheme 1: compound **2**)

To the solution of di-tert-butyl 2,2′-(1,4,7-triazonane-1,4-diyl) diacetate (**1**, 500 mg, 1.4 mmol) in anhydrous dichloromethane (20 mL) was added 200 μL of triethylamine (1.4 mmol) followed by 4-(bromomethyl)benzoic acid (310 mg, 1.4 mmol) in 5 mL anhydrous dichloromethane. The mixture was stirred at room temperature for 24 h. The mixture was washed with water, dried over sodium sulfate. The solvent was evaporated to produce the crude product and recrystallized from acetonitrile and diethyl ether to obtain 4-((4,7-bis(2-(tert-butoxy)-2-oxoethyl)-1,4,7-triazonan-1-yl)methyl)benzoic acid (**2**, 586 mg, 1.2 mmol, 86%) as a white solid.

$^{1}$H NMR (400 MHz, Acetonitrile $d_3$) $\delta$ 8.00 (d, $J$ = 7.9 Hz, 2H), 7.68 (d, $J$ = 8.0 Hz, 2H), 4.51 (s, 2H), 3.50–3.02 (m, 10H), 2.96–2.67 (m, 6H), 1.43 (s, 18H). $^{13}$C NMR (101 MHz, $CD_3CN$) $\delta$ 170.36, 166.46, 136.92, 132.24, 130.48, 130.02, 81.34, 58.22, 55.89, 50.89, 49.95, 47.55, 27.46.

MS (ESI) calculated mass for the parent $C_{26}H_{41}N_3O_6$, 492.31 $[M + H]^+$, found 492.30 $[M + H]^+$.

#### 2.1.2. Synthesis of 2,2′-(7-(4-((2,3,5,6-tetrafluorophenoxy)carbonyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA-Bz-TFPE, Scheme 2)

To the solution of **2** (586 mg, 1.2 mmol) in dioxane (30 mL) was added dicyclohexyl carbodiimide (DCC) (240 mg, 1.2 mmol) followed by 2,3,5,6-tetrafluorophenol (200 mg, 1.2 mmol). The mixture was stirred for 24 h and filtered. Solvent was evaporated under reduced pressure to produce di-tert-butyl 2,2′-(7-(4-((2,3,5,6-tetrafluorophenoxy)carbonyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetate (**3**). To the dichloromethane (5 mL) residue, 5 mL of trifluoroacetic acid was added. The solution was stirred