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New chemical and radiochemical routes to [^{18}F]Rho6G-DEG-F, a delocalized lipophilic cation for myocardial perfusion imaging with PET

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New chemical and radiochemical syntheses are described for the preparation of [^{18}F]Rho6G-DEG-F, an ^{18}F-labeled analogue of the fluorescent dye rhodamine 6G, which has shown promise as myocardial perfusion imaging agent. Tosylated precursors of [^{18}F]Rho6G-DEG-F amenable to ^{18}F-labeling were obtained either through a two-step synthesis from rhodamine 6G lactone (33% yield), or in one step from rhodamine 575 (64% yield), then purified by preparative C_{18} chromatography. Manual synthesis of [^{18}F]Rho6G-DEG-F was achieved in a single radiochemical step from either the tosylate salt or the tosylate/formate double salt in DMSO under standard nucleophillic aliphatic ^{18}F-fluorination conditions (K[^{18}F]F/K_2CO_3/Kryptofix 2.2.2.). Incorporation of the [^{18}F]F^- was found to be satisfactory (≥34% by TLC), despite the protic character of the precursor molecules. [^{18}F]Rho6G-DEG-F was manually synthesized in final decay-corrected radiochemical yields of 11–26% (tosylate salt) and 9–21% (tosylate/formate double salt). The protocol was transferred to an automated synthesis unit, where the product was obtained in 3–9% radiochemical yield (n = 3) decay corrected to start-of-synthesis, >99% radiochemical purity, and a molar activity of 122–267 GBq μmol^{-1} (3.3–7.2 Ci μmol^{-1}).

Introduction

Myocardial perfusion imaging (MPI) is an important component in the diagnosis and risk-stratification of coronary artery disease. Owing to its cost-effectiveness, readily available radiopharmaceuticals, and clinical utility, single-photon emission computed tomography (SPECT) MPI using ^{99m}Tc radiopharmaceuticals (*e.g.*, ^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin) remains a popular imaging technique.[1] However, there is currently considerable interest in the development of positron emission tomography (PET) radiopharmaceuticals for MPI because of the inherent advantages of PET in terms of spatial resolution, mitigation of attenuation artifacts, and the ability to accurately measure myocardial perfusion.[2] In this context, an ^{18}F-labeled MPI radiopharmaceutical is highly desirable because of the attractive nuclear properties of this radionuclide [*i.e.*, high positron yield (97%), low positron energy (β^+_{avg} = 250 keV), and intermediate half-life ($t_{1/2}$ = 109.8 min)] relative to other PET radionuclides used for MPI. For example, ^{82}Rb ($t_{1/2}$ = 1.3 min) is available from the ^{82}Sr/^{82}Rb decay pathway, but the generator is very expensive. Thus, high patient throughput is required to make this system cost-effective. [^{13}N]NH_3 is an effective MPI radiopharmaceutical, but the short half-life of ^{13}N ($t_{1/2}$ = 9.97 min) limits it use to facilities with an on-site cyclotron. To date, the most promising ^{18}F-labeled MPI candidate is [^{18}F]flurpiridaz, a pyridaben derivative that shows superior extraction fraction relative to all other MPI agents save [^{18}O]H_2O and is currently in a phase 3 clinical trial.[3–5] Other ^{18}F-labeled pyridaben derivatives are also being investigated for MPI,[6,7] as are a number of [^{18}F]fluoroalkyl[8–12] and [^{18}F]fluoroaryl[13] triphenylphosphonium salts.

Rhodamines are delocalized lipophilic cations (DLCs) that accumulate in mitochondria in proportion to mitochondrial membrane potential[14] and thus have been utilized extensively as potentiometric dyes[15] for the *ex vivo* study of cardiomyocytes.[16] With regard to ^{18}F-labeled rhodamines, we first synthesized rhodamine B derivatives bearing ^{18}F-alkyl[17] and ^{18}F-polyoxyethylene[18,19] esters and evaluated them for chemical and pharmacological parameters important to PET-MPI. Subsequently, AlJammaz *et al.* synthesized and

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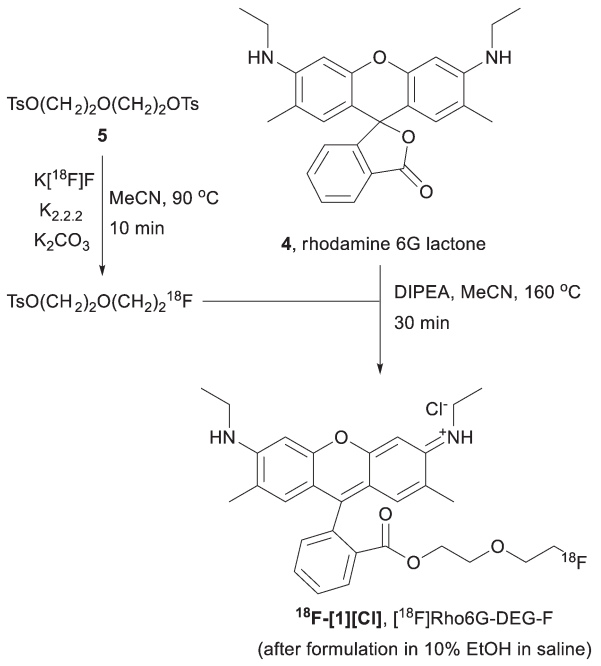
evaluated an [^{18}F]FDG-modified amide analogue of rhodamine 123[20] and Trencsényi *et al.* prepared 2-[^{18}F]fluoroethylrhodamine B for the *in vitro* measurement P-glycoprotein function.[21] Building on our earlier work with rhodamine B, we later compared the pharmacological properties of several 2-(2-[^{18}F]fluoroethoxy)ethyl ester derivatives bearing different rhodamine cores.[22] These ^{18}F-labeled rhodamine esters were prepared by condensing a rhodamine in the lactone form with the requisite ^{18}F/tosyl-bearing bifunctional prosthetic molecule. An example synthesis is shown in Scheme 1. On the basis of these experiments, rhodamine 6G 2-(2-[^{18}F]fluoroethoxy)ethyl ester, chloride salt ([^{18}F]Rho6G-DEG-F, **^{18}F-[1][Cl]**; Scheme 1) was identified as the most promising MPI candidate, exhibiting effective localization in isolated rat cardiomyocytes (4.6% ± 0.7% ID g^{-1} at 1 min), rapid and sustained uptake into rat hearts (1.3% ± 0.3% ID g^{-1} at 60 min), and excellent contrast between the heart and nearby liver tissues.[22] This two-step method for the preparation of **^{18}F-[1][Cl]** is not, however, optimal for routine production using automated synthesis modules primarily because the lactone precursor is sparingly soluble in many organic solvents, including MeCN. An additional limitation is that it requires two radiochemical steps. Thus clinical studies of [^{18}F]Rho6G-DEG-F are predicated on the development of an improved synthesis for this potential PET-MPI radiopharmaceutical, one that is amenable to use in automated synthesis systems. To this end, we now report the one-step synthesis of **^{18}F-[1][Cl]** by radiofluorination of the tosylate salt **[2][OTs]** and the double salt, **[2][HCOO][OTs]**, both of which can be prepared in a single step from commercially available starting materials.



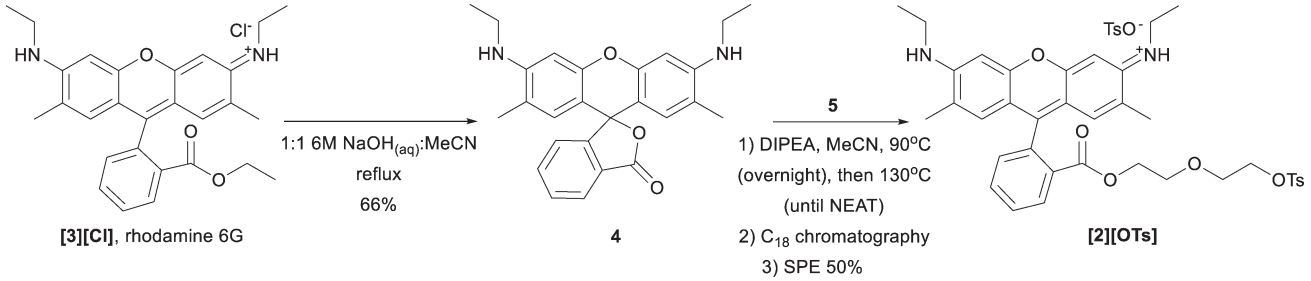
Scheme 1 Two-step radiosynthesis of [^{18}F]Rho6G-DEG-F.

Results and discussion

Two routes were developed for the synthesis of lipophilic cation [2]. The first was a two-step method, starting from the chloride salt of rhodamine 6G (**[3][Cl]**; Scheme 2). In this synthesis, a nucleophilic carboxylate anion is generated *in situ* by way of base-mediated hydrolysis of the ester, and the resultant zwitterion is converted to non-fluorescent lactone **4**. Formation of neutral **4** eliminates the chloride anion from the final product, thus removing it from potential competition with [^{18}F]F$^-$ during subsequent radiofluorination reactions. In the second synthetic step, **4** was suspended in acetonitrile and heated in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and diethylene glycol bis(*p*-toluenesulfonate) (**5**). The solvent and nitrogen base are gradually distilled off over time. The reaction presumably proceeds by way of zwitterion formation (**6**), followed by nucleophilic attack on **5** by the carboxylate anion. This general approach was first used for the preparation of modified rhodamine esters bearing carboxylic acid and benzyl chloride functionalities for conjugation to peptides,[23] and later for the synthesis of ^{19}F reference material **^{19}F-[1][Cl]**.[22] Cation [2] was purified on a glass column packed with C_{18} sorbent (50 : 50 MeCN : H_2O with 0.5% acetic acid), followed by solid-phase extraction (SPE) from the liquid chromatography (LC) eluent. The final product was determined to be the singly charged tosylate salt **[2][OTs]** (Scheme 2). The overall chemical yield of **[2][OTs]** using this two-step approach was 33%.

In the second route to precursor cation [2], commercially available inner salt rhodamine 575 (**6**) was esterified directly (Scheme 3). The product was purified on a preparative column of C_{18} sorbent using 45 : 55 MeCN : H_2O containing 0.1% formic acid as the mobile phase. In this method, however, the product salt was not extracted from the acidic LC eluent prior to concentration, which resulted in the formation of doubly charged **[2][HCOO][OTs]** as suggested by ^{1}H NMR and elemental analysis. Conventional understanding of K_2CO_3/Kryptofix 2.2.2. ($K_{2.2.2.}$) ^{18}F-fluorination chemistry suggests that this precursor may be inferior to **[2][OTs]** owing to the presence of an additional acidic proton that could interfere with fluoride nucleophilicity through the generation of H[^{18}F]F.[24] However, **[2][HCOO][OTs]** proved a serviceable precursor for standard nucleophilic aliphatic ^{18}F-fluorinations (*vide infra*). The isolated chemical yield of **[2][HCOO][OTs]** was 64%, approximately double the yield of [2] that was obtained using the two-step approach.

The utility of **[2][OTs]** and **[2][HCOO][OTs]** for the manual radiosynthesis of [^{18}F]Rho6G-DEG-F was verified using standard nucleophilic aliphatic ^{18}F-fluorination methods (Scheme 4). When the precursor salt was heated in anhydrous DMSO in the presence of the dried K[^{18}F]F/$K_{2.2.2.}$ complex and K_2CO_3, incorporation of [^{18}F]F$^-$ was found to be 34–47% (n = 3) and 34–69% (n = 7) by TLC for **[2][OTs]** and **[2][HCOO][OTs]**, respectively. These incorporation yields are somewhat surprising given the deleterious effect of Brønsted-Lowry acidic functionalities observed on many nucleophilic

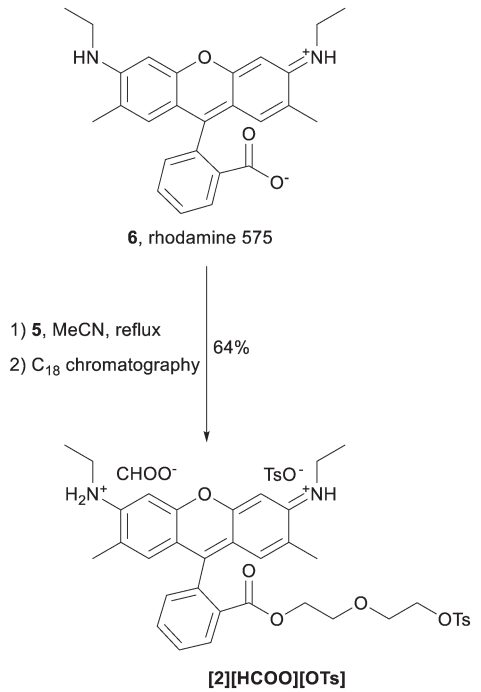


Scheme 2 Two-step synthesis of precursor **[2][OTs]**.

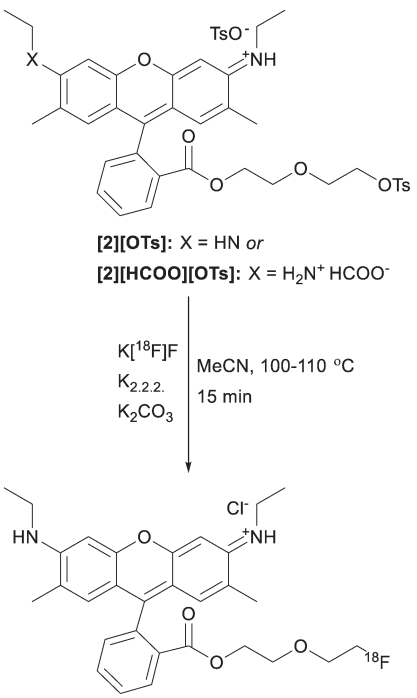
^{18}F-fluorination reactions. After reverse-phase semi-preparative HPLC, the product was isolated from the HPLC solvent by solid-phase extraction using a tC18 cartridge, concentrated to dryness, and formulated in 10% EtOH in isotonic saline.¶

Manual radiochemical yields of **^{18}F-[1][Cl]** from start-of-synthesis (SOS) were 11–26% (n = 3) decay-corrected (DC) and 9–21% (n = 6) DC for **[2][OTs]** and **[2][HCOO][OTs]** respectively. Molar activities were calculated based on a calibration curve prepared from an ^{19}F standard[22] (**[2][OTs]** = 3–9 GBq μmol^{-1}; **[2][HCOO][OTs]** = 7–35 GBq μmol^{-1}). These molar activity values are similar to those typically observed for manual ^{18}F syntheses carried out using small quantities (370–740 MBq) of [^{18}F]fluoride ion obtained from transfer line flushes after clinical production of other radiotracers. The fastest manual synthesis time was 97 min from SOS.

Finally, [^{18}F]Rho6G-DEG-F was prepared in an automated synthesis module (GE Tracerlab FX$_{FN}$) using conditions similar to those employed for the manual syntheses. As the yield and molar activity obtained using the two precursors were similar, **[2][OTs]** was employed as precursor for the automated synthesis. Decay-corrected radiochemical yields of 3, 7 and 9% (6 ± 3%) were achieved. The relatively low yields of **^{18}F-[1][Cl]** during automated syntheses are in part attributable to the inefficient extraction of [^{18}F]F$^-$ from Sep-Pak® Light QMA anion exchange cartridges (130 mg) using an elution matrix containing 0.9 mg mL^{-1} K_2CO_3 as the anion exchange reagent. Under these elution conditions, the non-decay-corrected (NDC) elution efficiency is only 50–65%.



Scheme 3 One-step synthesis of precursor **[2][HCOO][OTs]**.



Scheme 4 One-step radiosynthesis of [^{18}F]Rho6G-DEG-F.

¶ After preparative HPLC purification the radiotracer is assumed to be present as a TFA salt (**[1][TFA]**), which converts to the chloride salt (**[1][Cl]**) upon formulation in 10% EtOH in isotonic saline.

Furthermore, we observed that that the lipophilic ^{18}F-[1][Cl] product was partially retained by the PTFE tubing and the sterile filter used in the automated synthesis system. Despite these inefficiencies, the use of a remote synthesis apparatus allowed for the current "good manufacturing practices" (cGMP) production of 41, 56 and 81 GBq of [^{18}F]Rho6G-DEG-F and molar activities of 122, 180 and 267 (190 ± 73) GBq μmol^{-1}, respectively. The fastest automated synthesis time was 83 min from SOS.

Conclusions

Two synthetic routes were developed to the tosylated precursor of [^{18}F]Rho6G-DEG-F (^{18}F-[1][Cl]), a promising mitochondria-targeting ^{18}F tracer for PET-MPI. Despite being delocalized lipophilic salts, [2][OTs] and [2][HCOO][OTs] can be labeled with ^{18}F using conventional nucleophilic aliphatic ^{18}F-fluorination chemistry in a single radiochemical step. This facilitated the easy translation of this protocol to an automated synthesis system that will be used for the cGMP production of this radiopharmaceutical in upcoming human trials. Starting from precursor [2][OTs], ^{18}F-[1][Cl] was prepared in an automated synthesis unit in 6 ± 3% decay-corrected radiochemical yield from start-of-synthesis, high radiochemical purity (>99%), and high molar activity (190 ± 73 GBq μmol^{-1}).

Experimental

General information

Chemicals and media. Unless otherwise noted, reagents and solvents were purchased from Acros Organics or Alfa Aesar and were used without further purification. Rhodamine 575 (**6**; laser grade) was obtained from Organica (Bitterfeld-Wolfen, Germany). Diethylene glycol bis(*p*-toluenesulfonate) (**5**) was purchased from TCI America (Portland, OR, USA). Silica gel (40–63 μm) for flash chromatography was obtained from Silicycle, Inc. (Quebec City, Canada). LiChroprep® RP-18 sorbent (40–63 μm) was obtained from Merck KGaA. Quaternary methyl ammonium (QMA) anion-exchange cartridges (carbonate form) for manual [^{18}F]F$^-$ trapping and release were obtained from MedChem Imaging (Boston, USA). Sep-Pak® Light QMA cartridges (carbonate form) for automated synthesis were obtained from ABX (Radeberg, Germany). tC18 Light and C18 Plus Sep-Pak® solid phase extraction cartridges were obtained from Waters (Milford, MA, USA). Thin-layer chromatography (TLC) was performed on aluminum oxide coated polyethylene terephthalate sheets from Fluka (Ronkonkoma, NY, USA). Oxygen-18-enriched water (98% isotopic enrichment) was purchased from Rotem Industries Ltd. (Mishor Yamin, Israel).

Equipment

A Fujifilm BAS-5000 Phosphor Imager with Multi Gauge v3.0 software was used to assay TLC plates.

^{1}H and ^{13}C NMR spectra were recorded with a Varian 400-MR Spectrometer with a 400 MHz magnet. NMR solvents were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Chemical shifts (δ) are reported in ppm relative to the hydrogenated residue of the deuterated solvents. Elemental microanalysis (EA) was carried out by Atlantic Microlab, Inc. (Norcross, GA, USA).

HPLC systems described below, including data acquisition modules, are Shimadzu Prominence. The gamma detectors were optimized for 511 keV photons.

HPLC A. For manual preparations. Pump: LC-20AT. UV/vis detector: SPD-20A. Radiation detection: Carroll & Ramsey Model 105S.

HPLC B. Analytical. Pump: LC-20 AD. Diode array detector (DAD): SPD-M20A. Radiation detection: Harshaw NaI(Tl) detector with Canberra NIM electronics. For low resolution liquid chromatography-mass spectroscopy, an Advion expression^S CMS apparatus was added in-line.

HPLC C. For automated preparations. Pump: Sykam S1122. UV/vis detector: WellChrom Filter-Photometer K-2001. Radiation detection: GE FX_{FN} internal detector.

HPLC D. Analytical. Pump: LC-20AB. UV/Vis detector: SPD-20A Radiation Detection: Carroll & Ramsey Model 105S.

Program A. Column: EMD Millipore Purosphere® RP-18 endcapped, 5 μm, 4 mm × 125 mm. Solvent system: gradient elution, 10% MeCN in H_2O containing 0.1% formic acid for 2 min, raised to 90% MeCN in H_2O containing 0.1% formic acid over 13 min, hold for 5 min, flow rate = 1 mL min^{-1}. Detector: 190–800 nm (DAD).

Program B. Column: EMD Millipore Purosphere® RP-18 endcapped, 5 μm, 4 mm × 125 mm. Solvent system: gradient elution, 10% MeCN in H_2O containing 0.1% trifluoroacetic acid (TFA) for 2 min, raised to 90% MeCN in H_2O containing 0.1% TFA over 13 min, hold for 5 min, flow rate = 1 mL min^{-1}. Detector: 190–800 nm (DAD).

Program C. Column: ES Industries Chromegabond WR C18, 5 μm, 120 Å, 9.6 mm × 250 mm. Solvent system: isocratic elution, 50 : 50 MeCN : H_2O containing 0.1% TFA, flow rate = 3 mL min^{-1}. Detector: 254 nm.

Program D. Column: EMD Millipore Purosphere® RP-18 endcapped, 5 μm, 4 mm × 125 mm. Solvent system: isocratic elution, 50 : 50 MeCN : H_2O containing 0.1% TFA, flow rate = 1 mL min^{-1}. Detector: 190–800 nm (DAD).

Program E. Column: Phenomenex Luna C18, 10 μm, 100 Å, 10 mm × 250 mm. Solvent system: isocratic elution, 45:55 MeCN: citrate buffer (0.1 M, pH = 2.7), flow rate = 5 mL min^{-1}. Detector: 254 nm.

Program F. Column: Phenomenex Luna C18, 5 μm, 100 Å, 4.6 mm × 250 mm. Solvent system: isocratic elution, 50 : 50 MeCN : H_2O containing 0.1% TFA, flow rate = 1 mL min^{-1}. Detector: 254 nm.

Non-radioactive chemistry

Synthesis of rhodamine 6G lactone (4). Rhodamine 6G ([3][Cl]; 4.4 g, 9.2 mmol) was dissolved in MeCN (30 mL). To this solution 6 M NaOH (30 mL) was added with stirring, and the reaction mixture was refluxed overnight. After cooling to

room temperature, the reaction mixture was extracted with 3 × 50 mL chloroform. The organic layers were combined and dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated *in vacuo.* The crude product was further purified on a flash column of silica gel. The column was eluted first with 3% triethylamine (TEA) in 6 : 4 CH_2Cl_2 : hexanes, followed by a step-wise decrease in hexanes content until all visible impurities were eluted. The product was then eluted from the column with 8 : 2 CH_2Cl_2 : EtOH containing 3% TEA. The fractions containing the product were pooled and concentrated *in vacuo*, with the addition of 3 × 10 mL of EtOH added to facilitate the removal of TEA. After drying under high vacuum, 2.5 g (66%) of **4** was obtained as a pink powder. The identity and purity of lactone **4** was verified by ^{1}H NMR[23] and LC-MS (**HPLC B**, **Program A**, t_R = 10.3 min, *m*/*z* 415 $[M+H]^+$).

Synthesis of rhodamine precursor [2][OTs]. To a round-bottom flask containing rhodamine 6G lactone (**4**) (1.66 g, 4.0 mmol) in MeCN (30 mL), diethylene glycol bis(*p*-toluenesulfonate) (**5**; 3.32 g, 8.0 mmol) and DIPEA (3 mL, 18 mmol) were added. The flask was capped with a rubber stopper punctured with a vent needle and the reaction mixture was heated to 90 °C overnight with stirring, during which time the solvent partially evaporated. The next morning, the reaction was heated to 130 °C and held there until all remaining solvent was removed. The flask was then cooled to room temperature, and the crude product (a purple oil) was purified on a glass column packed with C_{18} sorbent (LiChroprep® RP-18). The column was eluted by gravity with 50 : 50 MeCN : H_2O containing 0.5% acetic acid as the mobile phase. The collected fractions were pooled and diluted with water (200 mL), then a fraction of this solution (~1/3) was passed through two solid-phase extraction cartridges placed in-line [Sep-Pak® C18 Plus, activated previously with EtOH (5 mL) and water (20 mL)]. This step was repeated twice more with the remaining fractions. The product was eluted from the three sets of cartridges with EtOH (5 mL each) and concentrated under reduced pressure to afford **[2][OTs]** as a purple solid (1.65 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (t, J = 8.0, 6H), 2.19 (s, 6H), 2.31 (s, 3H), 2.41 (s, 3H), 3.32–3.50 (m, 8H), 4.05 (m, 4H), 6.50 (s, 2H), 6.68 (s, 2H), 7.12 (d, J = 8.0, 2H), 7.27–7.40 (m, 5H), 7.73–7.82 (m, 6H), 8.35 (dd, J = 1.2, 7.8, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.65, 17.95, 21.27, 21.62, 38.47, 64.20, 68.43, 68.73, 68.99, 93.48, 113.18, 126.05, 126.22, 127.84, 128.23, 128.40, 129.75, 129.90, 131.60, 132.73, 133.01, 134.22, 138.86, 143.91, 145.04, 156.03, 156.27, 156.94, 157.69, 164.98. LC-MS (**HPLC B**, **Program A**, t_R = 12.0 min, *m*/*z* 657 $[M]^+$. Anal. calcd. for $C_{44}H_{48}N_2O_{10}S_2$: C, 63.75; H, 5.84; N, 3.38%. Found: C, 63.95; H, 5.83; N, 3.51%.

Synthesis of rhodamine precursor [2][HCOO][OTs]. Into a round-bottomed flask containing diethylene glycol bis(*p*-toluenesulfonate) (**5**; 2.50 g, 6.0 mmol) in dry MeCN (20 mL) was added rhodamine 575 (**6**; 0.50 g, 1.2 mmol), in portions, with stirring. The reaction was heated to reflux for 21 h. Reaction monitoring was carried out using neutral alumina TLC (10% EtOH in $CHCl_3$). Upon cooling to room temperature, a precipitate formed. The reaction mixture was diluted with additional MeCN (20 mL) and centrifuged for 10 min (13 000 rpm). The solvent was decanted off and concentrated *in vacuo* to form a viscous maroon oil which crystallized upon standing. The product was purified by preparative C_{18} chromatography. Elution of the column with 45 : 55 MeCN : H_2O containing 0.1% formic acid followed by concentration of the collected fractions at reduced pressure yielded tosylate/formate double salt **[2][HCOO][OTs]** (619 mg, 64%) as a glassy red solid. ^{1}H NMR (400 MHz, CD_2Cl_2): δ 1.37 (t, J = 7.2 Hz, 6H), 2.15 (s, 6H), 2.32 (s, 3H), 2.41 (s, 3H), 3.31–3.37 (m, 2H), 3.43–3.54 (m, 6H), 3.98–4.05 (m, 4H), 6.49 (br s, 2H), 6.71 (s, 2H), 6.82 (d, J = 0.9 Hz, 2H), 7.13 (d, J = 7.9 Hz, 2H), 7.29–7.37 (m, 3H), 7.67–7.75 (m, 3H), 7.75–7.85 (m, 2H), 8.25 (s, 1H), 8.34 (dd, J = 1.4, 7.8 Hz, 1H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 14.22, 17.85, 17.86, 21.55, 21.57, 21.94, 21.96, 39.29, 64.81, 69.00, 69.28, 69.74, 94.42, 94.44, 114.37, 126.17, 126.45, 128.34, 129.06, 129.41, 129.42, 130.42, 130.48, 130.71, 130.77, 132.06, 133.37, 133.52, 134.52, 139.95, 139.97, 145.84, 156.61, 157.88, 158.48, 162.85, 165.47. LC-MS (**HPLC B**, **Program A**, t_R = 12.0 min, *m*/*z* 657 $[M]^+$). Anal. calcd. for $C_{45}H_{50}N_2O_{12}S_2$: C, 61.77; H, 5.76; N, 3.20%. Found: C, 61.96; H, 5.94; N, 3.40%.

Radiochemistry

Preparation of [^{18}F]fluoride ion. No-carrier-added [^{18}F]fluoride ion was produced by proton bombardment of 3.5 mL of [^{18}O]water [^{18}O(*p*,*n*)^{18}F reaction] on the GE 16.5 MeV PETtrace cyclotron at Brigham and Woman's Hospital BICOR facility. An estimated 96 GBq activity was produced at EOB for automated syntheses. For manual experiments, 740–1850 MBq of [^{18}F]fluoride ion from an earlier production run was used.

Example radiosyntheses of [^{18}F]Rho6G-DEG-F (^{18}F-[1][Cl])

Manual radiosynthesis. [^{18}F]Fluoride ion was extracted from [^{18}O]H_2O *via* immobilization on a QMA anion-exchange cartridge (carbonate form; 10–12 mg; MedChem Imaging), which was previously activated with water (1 mL). The activity was eluted from the sorbent into a glass conical vial (2 mL) with a solution of K_2CO_3 (0.5 mg) in water (0.2 mL), followed by $K_{2.2.2.}$ (2.5 mg) in dry MeCN (0.8 mL). The extraction efficiency of this step was 90% NDC. Solvent was removed by azeotropic distillation at 110 °C under an argon stream. An additional portion of dry MeCN (1 mL) was added, and a second evaporation step was carried out at 110 °C. In the same fashion a third evaporation step was carried out. The evaporation steps took 15 minutes total. The reaction vessel was cooled by partially submerging it in tap water, then the precursor compound **[2][HCOO][OTs]** (0.5 mg) in anhydrous DMSO (0.5 mL) was added. The vial was sealed and heated to 110 °C for 15 min. After cooling, a portion was removed for radio-TLC (neutral alumina, 10% MeOH in CH_2Cl_2) and analytical HPLC (**HPLC B**, **Program B**, t_R = 12.6 min), then the bulk mixture was quenched with an aqueous solution of 0.1% TFA (500 μL). After semi-preparative HPLC purification (**HPLC A**, **Program C**, t_R = 22.5 min), the collected product¶ was diluted with water (50 mL) and trapped on a Sep-Pak®

Light tC18 SPE cartridge that was previously activated with EtOH (10 mL) and water (10 mL). **^{18}F-[1][TFA]** was eluted from the cartridge with EtOH (1 mL), and the eluate was evaporated at 110 °C. The final product was formulated in 10% EtOH in 0.9% saline (1 mL) and assayed by analytical HPLC (**HPLC B, Program D**, t_R = 6.7 min). Non-decay corrected preparative yield of **^{18}F-[1][Cl]** was 11% (21% DC) from SOS. Total synthesis time was 97 min from SOS.

Automated radiosynthesis. [^{18}F]Fluoride ion in [^{18}O]H_2O was transferred to a GE Tracerlab FX_{FN} automated radiosynthesis module where the [^{18}F]fluoride ion was immobilized on a Sep-Pak® Light QMA cartridge (ABX). [^{18}F]Fluoride ion was partially eluted into a reaction vessel using a mixture of $K_{2.2.2.}$ (5 mg) in dry MeCN (0.9 mL) and K_2CO_3 (0.9 mg) in H_2O (0.1 mL) providing approximately 81 GBq of activity (65% NDC efficiency). The extraction solvent was heated to 60 °C under a continuous stream of helium, and the temperature was held there for 7 min. Next, the temperature was increased to 120 °C to complete the azeotropic distillation process, followed by immediate cooling of the reaction vessel to 50 °C. Precursor **[2][OTs]** (1 mg) in DMSO (1 mL) was added and the reaction mixture was heated to 100 °C for 30 min. Upon completion of the reaction, the crude product was diluted with 3.5 mL of HPLC mobile phase and injected onto a semi-preparative HPLC column (**HPLC C, Program E**, t_R = ~18 min). **^{18}F-[1][TFA]** was collected and diluted with water (50 mL), followed by trapping on a Sep-Pak® C18 Plus SPE cartridge (previously conditioned with 5 mL EtOH and 10 mL water). The tracer was eluted from the cartridge using EtOH (1 mL), followed by 0.9% saline (5.5 mL). This solution was then passed through a 0.2 μm sterile filter and collected in a vented vial. An additional aliquot of saline (3.5 mL) was passed through the filter giving 6.6 GBq (5.3% NDC, 9.0% DC yield from SOS) of the injectable final product, **^{18}F-[1][Cl]**, in 10% EtOH in saline. The identity of the formulated compound was confirmed by analytical HPLC (**HPLC D, Program F**, t_R = 11.3 min). Total automated synthesis time was 86 min from SOS.

Conflict of interest

The authors declare no competing interests.

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Notes and references

1 *Myocardial Perfusion Imaging (Revised Edition): A Technologist's Guide*, ed. H. Ryder, G. Testanera, V. Veloso Jerónimo and B. Vidovič, European Association of Nuclear Medicine, Vienna, 2014.
2 M. Salerno and G. A. Beller, *Circ. Cardiovasc. Imaging*, 2009, **2**, 412–424.
3 D. S. Berman, G. Germano and P. J. Slomka, *J. Nucl. Cardiol.*, 2012, **19 Suppl 1**, S38–45.
4 J. Maddahi, J. Czernin, J. Lazewatsky, S. C. Huang, M. Dahlbom, H. Schelbert, R. Sparks, A. Ehlgen, P. Crane, Q. Zhu, M. Devine and M. Phelps, *J. Nucl. Med.*, 2011, **52**, 1490–1498.
5 D. S. Berman, J. Maddahi, B. K. Tamarappoo, J. Czernin, R. Taillefer, J. E. Udelson, C. M. Gibson, M. Devine, J. Lazewatsky, G. Bhat and D. Washburn, *J. Am. Coll. Cardiol.*, 2013, **61**, 469–477.
6 T. Mou, Z. Zhao, P. Zhang, W. Fang, C. Peng, J. Lu, Q. Wang, Y. Ma and X. Zhang, *Chem. Biol. Drug Des.*, 2015, **86**, 351–361.
7 T. Mou, Z. Zhao, W. Fang, C. Peng, F. Guo, B. Liu, Y. Ma and X. Zhang, *J. Nucl. Med.*, 2012, **53**, 472–479.
8 H. T. Ravert, I. Madar and R. F. Dannals, *J. Labelled Compd. Radiopharm.*, 2004, **47**, 469–476.
9 I. Madar, H. Ravert, A. Dipaula, Y. Du, R. F. Dannals and L. Becker, *J. Nucl. Med.*, 2007, **48**, 1021–1030.
10 I. Madar, H. T. Ravert, Y. Du, J. Hilton, L. Volokh, R. F. Dannals, J. J. Frost and J. M. Hare, *J. Nucl. Med.*, 2006, **47**, 1359–1366.
11 L. Li, L. Brichard, L. Larsen, D. K. Menon, R. A. J. Smith, M. P. Murphy and F. I. Aigbirhio, *J. Labelled Compd. Radiopharm.*, 2013, **56**, 717–721.
12 A. Haslop, L. Wells, A. Gee, C. Plisson and N. Long, *Mol. Pharmaceutics*, 2014, **11**, 3818–3822.
13 Z. Cheng, M. Subbarayan, X. Chen and S. S. Gambhir, *J. Labelled Compd. Radiopharm.*, 2005, **48**, 131–137.
14 R. C. Scaduto and L. W. Grotyohann, *Biophys. J.*, 1999, **76**, 469–477.
15 G. Solaini, G. Sgarbi, G. Lenaz and A. Baracca, *Biosci. Rep.*, 2007, **27**, 11–21.
16 E. N. Dedkova and L. A. Blatter, *J. Mol. Cell. Cardiol.*, 2012, **52**, 48–61.
17 T. K. Heinrich, V. Gottumukkala, E. Snay, P. Dunning, F. H. Fahey, S. Ted Treves and A. B. Packard, *Appl. Radiat. Isot.*, 2010, **68**, 96–100.
18 M. D. Bartholoma, V. Gottumukkala, S. Zhang, A. Baker, P. Dunning, F. H. Fahey, S. T. Treves and A. B. Packard, *J. Med. Chem.*, 2012, **55**, 11004–11012.
19 M. D. Bartholoma, H. He, C. A. Pacak, P. Dunning, F. H. Fahey, F. X. McGowan, D. B. Cowan, S. T. Treves and A. B. Packard, *Nucl. Med. Biol.*, 2013, **40**, 1043–1048.
20 I. AlJammaz, B. Al-Otaibi, H. AlHindas and S. M. Okarvi, *Nucl. Med. Biol.*, 2015, **42**, 804–808.
21 G. Trencsényi, I. Kertesz, Z. T. Krasznai, G. Mate, G. Szaloki, P. Szabo Judit, L. Karpati, Z. Krasznai, T. Marian and K. Goda, *Eur. J. Pharm. Sci.*, 2015, **74**, 27–35.
22 M. D. Bartholoma, S. Zhang, V. Akurathi, C. A. Pacak, P. Dunning, F. H. Fahey, D. B. Cowan, S. T. Treves and A. B. Packard, *Nucl. Med. Biol.*, 2015, **42**, 796–803.
23 C. A. M. Afonso, V. Santhakumar, A. Lough and R. A. Batey, *Synthesis*, 2003, 2647–2654, DOI: 10.1055/s-2003-42475.
24 H. H. Coenen, in *PET chemistry: the driving force in molecular imaging*, ed. A. Schubiger, L. Lehmann and M. Friebe, Springer, Berlin, New York, 2007, xii, p. 339.

