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High-Yielding Aqueous ^{18}F -Labeling of Peptides via Al^{18}F chelation

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Abstract

The coordination chemistry of a new pentadentate bifunctional chelator (BFC), NODA-MPAA **1**, containing the 1,4,7-triazacyclononane-1,4-diacetate (NODA) motif with a methyl phenyl acetic acid (MPAA) backbone, and its ability to form stable Al^{18}F -chelates, was investigated. The organofluoroaluminates were easily accessible from the reaction of **1** and AlF_3 . X-ray diffraction studies revealed aluminum at the center of a slightly distorted octahedron, with fluorine occupying one of the axial positions. The *tert*-butyl protected prochelator **7**, which can be synthesized in one step, is useful for coupling to biomolecules on solid phase or in solution. High yield (55–89%) aqueous ^{18}F -labeling was achieved in 10–15 minutes with a tumor-targeting peptide **4** covalently linked to **1**. Defluorination was not observed for at least 4 h in human serum at 37 °C. These results demonstrate the facile application of Al^{18}F chelation using BFC **1** as a versatile labeling method for radiofluorinating other heat-stable peptides for positron emission imaging.

INTRODUCTION

Molecular imaging is the non-invasive visualization of cellular or molecular processes that promises to improve the diagnosis and monitoring of various diseases or conditions.^{1,2} The most commonly used molecular imaging modalities include fluorescence, bioluminescence, positron-emission tomography (PET), and single-photon emission computed tomography (SPECT).³ PET has emerged as a modality of choice, because it yields images with good spatial resolution and excellent sensitivity.^{4,5} Among the positron-emitting isotopes (^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{68}Ga , ^{89}Zr , ^{124}I), ^{18}F has the advantages of suitable decay properties ($t_{1/2}$ = 109.7 min, ~ 97% β^+ -emission, 635 keV), low β^+ -trajectory (<2 mm), small atomic size, and is readily produced from a stable heavy atom (^{18}O) precursor.^{6,7} Currently, one of the most widespread applications of PET/CT is the measurement of glucose metabolism using ^{18}F -fluorodeoxyglucose (^{18}F -FDG).⁸ Radiofluorination traditionally has involved C- ^{18}F bond formation that is performed in anhydrous polar aprotic solvents at 80–160 °C via $\text{S}_{\text{N}}2$ or $\text{S}_{\text{N}}\text{Ar}$ mechanism.^{9–11} Although the aryl C- ^{18}F bonds are generally stable *in vivo*, the aliphatic C- ^{18}F is often prone to enzymatic cleavage. C- ^{18}F bonds have also been

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Supporting Information Available. HPLC chromatograms, crystallographic data and CIF files. This information is available free of charge via the Internet at <http://pubs.acs.org>.

introduced by the direct electrophilic addition of molecular fluorine ($^{18}\text{F}_2$) across double bonds.¹² New radiolabeling chemistries, utilizing the fluorides of phosphorus, silicon, and boron have been explored.^{13–15}

We previously reported a technique that exploits the fluorophilic nature of aluminum to afford direct aqueous ^{18}F -labeling by the formation of stable aluminum-fluoride chelates.¹⁶ Initial studies involved two chelation systems, the acyclic BFC diethylenetriaminepentaacetic acid (DTPA) and the macrocyclic BFC 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA). While the former had excellent binding kinetics, its complexes were not stable, being prone to hydrolysis and demetalation in serum, whereas the NOTA derivative formed highly stable Al^{18}F complexes.¹⁶

The hexadentate *p*-SCN-Bn-NOTA was used to synthesize IMP449 (Figure 1), a hapten-peptide for *in vivo* targeting of cancer with a bispecific antibody (bsMAb) pretargeting system, a highly sensitive and specific technique for localizing cancer.^{17–19} Although the Al^{18}F (IMP449) was stable *in vivo* and displayed excellent tumor to blood (T/B) ratios, radiochemical yields (RCYs) were low (5–20%).¹⁶ This could be due to the participation of all three *N*-acetate arms of the N_3O_3 donor set in the complexation, resulting in a restricted coordination site for binding ^{18}F . To address this issue, we synthesized IMP461 (Figure 1), a similar hapten-peptide with NOTA attached via one of its *N*-acetates, and obtained stable complexes, indicating that it was possible to diminish denticity without significant loss of ligand effectiveness.^{20,21} This prompted us to focus on pentadentate derivatives of 1,4,7-triazacyclononane (TACN) that could form mononuclear octahedral complexes with aluminum and possess a single coordination site for binding fluorine. Herein, we report the coordination chemistry of a new pentadentate ligand **1**, containing the 1,4,7-triazacyclononane-1,4-diacetate motif, and its ability to form physiologically stable Al^{18}F -labeled chelates when covalently linked to a hapten-peptide (IMP485) **4** via an amide bond (Figure 2). These results establish the broad applicability of this chemical strategy in the development of ^{18}F -labeled biomolecules useful for PET imaging.

RESULTS

The reaction of **1** (Figure 2) with Al^{3+} was studied in the presence of $^{19}\text{F}^-$. Reacting equimolar amounts of AlCl_3 and **1** at 104 °C for 10 min yields a polar chelate **2** with retention time (t_R) 8.98 min (Figure 3A). With equimolar amounts of NaF, AlCl_3 and **1**, the Al^{19}F complex **3** (13.8%) was obtained, with t_R 12.29 min (Figure 3B), along with **2** and unreacted **1**. Since AlCl_3 and NaF readily react²² to produce $\text{AlF}_n(3-n)^+$, we investigated the reaction between AlF_3 and **1**. Although the AlF_3 remained as a fine suspension in the 2 mM AlF_3 solution, we reacted it with **1** at 105 °C for 10 min and obtained a low yield (4.3%) of **3** (Figure 3C). Suspecting that a hydrophilic organic solvent might better solubilize AlF_3 , we carried out the reaction in the presence of 50 μL methanol, which increased the yield of **3** to 27.7% (Figure 3D). Subsequent experiments revealed that the presence (50% or more v/v) of other hydrophilic organic solvents (e.g., acetonitrile, ethanol, *n*-propanol, isopropanol, *t*-butyl alcohol, acetone, dioxane, tetrahydrofuran, dimethylsulfoxide, or dimethyl formamide) also improved the yield of **3**.

The next study determined if fluoride, which is bioisosteric with both the hydrogen atom and the hydroxyl group, could displace the hydroxide from **2**. When equimolar amounts of **2** and NaF were reacted at 100 °C for 10 min, a mixture containing **3** (32.3%) along with **1** and **2** (Figure 3E) was obtained, indicating a possible substitution mechanism, accompanied by demetalation. When the above reaction was performed in the presence of ethanol, we obtained a two-fold increase in the yield of **3** (Figure 3F).

To determine their stability and structural features, **2** and **3** were synthesized by reacting **1** with AlCl_3 or AlF_3 , respectively (Scheme 1). When the Al^{19}F chelate **3** was incubated in PBS, pH 7.4, at 37 °C, no detectable loss of aluminum was observed by RP- HPLC, after 24 h. However, the corresponding Al-OH complex **2** was not as stable under the same conditions, undergoing progressive demetalation (e.g., 23% in 3 h, 61% in 12 h; see Supporting Information, Table S1).

The HRMS of **1** $(\text{M}+\text{H})^+$ 394.20 and that of **3** with $(\text{M}+\text{H})^+$ 438.16 confirms the presence of an aluminum-fluoride complex (see Supporting Information, Figure S1). Mass spectrum of **2** has molecular ion peak $(\text{M}+\text{H})^+$ 436.17 of low abundance, the parent peak being 418.16, molecular ion minus hydroxide, indicating that the sixth coordination site is occupied by hydroxide instead of chloride.

In the uncomplexed BFC **1**, the $\text{N-CH}_2\text{-Ph}$ and $\text{Ph-CH}_2\text{-COOH}$ protons appear as singlets at δ 4.32 and δ 3.61 (Figure 4A). Upon coordination with aluminum fluoride, the $\text{N-CH}_2\text{-Ph}$ protons are split into a pair of doublets at δ 4.24 and δ 3.74 ppm. The region from δ 2.24 to 3.51 ppm is crowded, with several overlapping multiplets arising due to the splitting of the 12 $\text{N-CH}_2\text{-CH}_2\text{-N}$ ring and 4 $\text{N-CH}_2\text{-COO}^-$ protons, along with dissolved water (δ 3.3 ppm) molecules in DMSO-d_6 (Figure 4B). These multiplet patterns arise from ring conformational interconversions, which are not so rapid to hinder isomer detection by NMR spectroscopy.²³

Mono-hydrated single crystals of **3** suitable for X-ray crystallography were grown from a concentrated solution of acetonitrile containing traces of water. The intensity data for **3** were measured on a Bruker-Nonius KappaCCD diffractometer (graphite- monochromated $\text{Mo K}\alpha$ radiation, $\lambda = 0.71073 \text{ \AA}$, φ - ω scans) at 100 (1) K. The data were corrected for absorption. The single-crystal X-ray crystallography (Figure 5) reveals that **3** crystallizes with approximate dimensions $0.060 \times 0.080 \times 0.52 \text{ mm}$ and were monoclinic with space group $P2_1/c$. The final unit-cell constants of **3** were $a = 20.463(4) \text{ \AA}$, $b = 8.495(2) \text{ \AA}$, $c = 12.692(3) \text{ \AA}$, $\beta = 107.52(3)^\circ$, $V = 2103.9(7) \text{ \AA}^3$, $Z = 4$, $\rho = 1.438 \text{ g cm}^{-3}$, $\mu = 0.15 \text{ mm}^{-1}$, formula weight = 1821.66. The structure of **3** was solved with SHELXS-97 and refined by full-matrix least squares on F^2 with SHELXL-97. The hydrogen atoms were calculated with the riding model in the structure-factor calculations, but their parameters were not refined. The final discrepancy indices, $2.93 < \theta < 27.48^\circ$, were $R = 0.0601$ (calculated on F for 3241 reflections) and $R_w = 0.1374$ (calculated on F^2 for all 4812 reflections) with 281 parameters varied. The major peaks of the final difference map, -0.39 and $+0.32 \text{ e \AA}^{-3}$, are near the aluminum atom. For crystallographic data (Table S2) and CIF files, see Supporting Information.

We next turned to examining radiolabeling yields using $^{18}\text{F}^-$ in saline. When a mixture of NODA-MPAA **1** (10 μL , 20 nmol), AlCl_3 (5 μL , 10 nmol) and 40 μL $^{18}\text{F}^-$ (1.326 mCi) in 0.9% saline were reacted at 101 °C for 15 min, decay corrected RCY post purification by solid-phase extraction (SPE) based on $^{18}\text{F}^-$ was 6.9%. With 50 μL ethanol as co-solvent, RCY increased to 52.8%, validating our results with $^{19}\text{F}^-$. When the Al-OH chelate **2** (10 μL , 20 nmol) was reacted with 45 μL $^{18}\text{F}^-$ in 0.9% saline at 101 °C for 15 min, in the absence and presence of ethanol, decay corrected RCYs were 29.6% and 63.9%, respectively.

Having established the suitability of NODA-MPAA for radiofluorination, the next studies focused on the radiolabeling of the NODA-MPAA pretargeting hapten-peptide, IMP485 **4**. Under the conditions shown in Table 1, RCYs increased from ~24% in aqueous conditions, to 75–90% in the presence of ethanol and increasing amounts of Na^{18}F . A two-fold increase in RCYs was also observed in the presence of other hydrophilic organic solvents (Table 2). The Radio-HPLC chromatogram (Method 2) of the SPE-purified peptide $\text{Al}^{18}\text{F}(\text{IMP485})$

shows a single peak with t_R 15.789 min (Figure 6A), while the cold standard $Al^{19}F(IMP485)$ appears at 15.620 min (Figure 6B). There was no evidence of defluorination when $Al^{18}F(IMP485)$ was incubated in human serum at 37 °C for 4 h (see Supporting Information, Figure S3). Efficient radiofluorination (decay corrected RCY 72.3%, 1.28 Ci/ μ mol) was observed when a solution of $AlOH(IMP485)$ **5** (10 μ L, 20 nmol), 110 μ L ethanol and 100 μ L $^{18}F^-$ (43.3 mCi) in 0.9% saline were reacted at 105 °C for 15 min (Table 3). Under identical conditions, labeling efficiency of IMP485 **4**, was consistently higher than that observed with the NOTA- derived hapten-peptides IMP449 and IMP461 (see Supporting Information, Table S3–S5).

DISCUSSION

With the availability of compact cyclotrons, automated chemical modules and microreactors, PET imaging using ^{18}F has become the modality of choice for molecular imaging of many small molecules.²⁴ Almost 90% of all clinical PET imaging is performed using ^{18}F -FDG, the other 10% includes agents like ^{18}F -NaF used for bone imaging; ^{18}F -FLT, ^{18}F -choline, and ^{18}F -FET in oncology; and ^{18}F -fallypride, ^{18}F -DOPA, and ^{18}F -altanserine in neurosciences.²⁵ Despite its popularity, FDG cannot be used for many neurological, oncological and cardiological conditions, making it imperative to seek other compounds.²⁶ There are numerous receptor-avid biomolecules, like folate, biotin, bombesin (BBN), gastrin-releasing peptide (GRP), RGD, somatostatin, antibodies, peptides, and proteins, which possess high specificity and affinity.^{27,28} Development of a facile method of tagging these molecules with ^{18}F would enhance their utility in the detection of pathologically distinct cellular targets. Most ^{18}F -labeling methods are tedious to perform and require the efforts of specialized chemists. Multiple purifications of intermediates are commonly required, resulting in low RCYs. Additionally, ^{18}F -labeling involving C- ^{18}F bond formation, which is employed in all clinically-approved ^{18}F -labeled products, requires anhydrous conditions.²⁹ The newer labeling strategies, involving B- ^{18}F and Si- ^{18}F bond formation, have shown promise, but suffer from the disadvantage of rather high lipophilicity of the building block,^{30,31} a property that can enhance hepatobiliary clearance *in vivo*. An ideal method is one in which a peptide could be labeled rapidly in the final step with high specific activity in an aqueous medium.

With experience gained in rapid and quantitative binding of various radiometals with chelates, and with the knowledge that $^{18}F^-$ avidly binds to Al^{3+} , we initiated a project to determine whether a stable $Al^{18}F$ complex could be formed with peptides coupled with an appropriate BFC. Our initial studies were performed with peptides covalently linked to the hexadentate *p*-SCN-Bn-NOTA and NOTA (Figure 1) via one of its *N*-acetates.^{16,21} These peptides yield highly stable $Al^{18}F$ -chelates (*in vitro* and *in vivo*), but with low RCYs. We rationalized that with a hexadentate ligand, one of the pendant carboxylates might not participate in the complexation, and the presence of uncoordinated donor atoms potentially could impose steric constraints on the coordinated ligand framework, resulting in isomerism and/or instability. We then set out to synthesize a BFC that could form mononuclear octahedral complexes with aluminum, leaving a vacant site for binding fluorine. Since TACN-derived BFCs are suitable for Al^{3+} (0.53 Å), we decided to use NO2ArBu with two pendant *N*-acetate arms as our building block. BFCs with pendant *N*-acetates are common, since they do not affect the molecular weight significantly and their high hydrophilicity favors rapid renal clearance. The pentadentate NODA-MPAA **1**, with its N_3O_2 donor set, was chosen because the *tert*-butyl protected prochelator **7** can be synthesized by one simple alkylation (Scheme 2). The methyl phenyl acetic acid (MPAA) backbone acts as a spacer and makes for easy linkage to biomolecules on solid phase or in solution.

The X-ray structure (Figure 5) confirms the ability of **1** to form an Al¹⁹F monohydrated complex, with the MPAA backbone distanced from the coordination sphere. The complex is neutral with aluminum located at the center of a slightly distorted octahedron, as evidenced by the deviation of the twelve quasi-orthogonal and three linear bond angles, from the ideal angles of 90 and 180° in a regular octahedron. The average of these twelve quasi-orthogonal angles and that of the three linear bond angles are 89.89° and 169.60°, respectively (Table 4). The two TACN ring nitrogens and two oxygens, one from each pendant carboxylate, lie in the equatorial plane. One axial plane is occupied by the third nitrogen of TACN, while the fluorine lies in the opposite axial position, completing the octahedral coordination sphere. The diversity of the bond distances between the aluminum and the hetero atoms N1, N4, N7, O12, O14, and F also contributes to the asymmetry of the aluminum coordination sphere. The Al-N4 bond is significantly shorter (2.036 Å) compared to the Al-N1 (2.074 Å) and Al-N7 (2.080 Å) bonds, while the Al-O14 (1.875 Å) and Al-O12 (1.854 Å) bond lengths are also slightly different. The longer Al-N1 (2.074 Å) bond could be a reflection of the high affinity of the *trans* fluoro ligand for aluminum. In comparison with the crystallized Al(NOTA), with Al-N and Al-O bond lengths of 2.06 Å and 1.84 Å, respectively,³² the small difference in the average Al-N (2.063 Å) and Al-O (1.865 Å) bond lengths in **3** indicates that aluminum is strongly coordinated by the N₃O₂ donor set.

When incubated in PBS, pH 7.4, at 37 °C, Al¹⁹F(NODA-MPAA) **3** was stable for over 24 h, while the corresponding Al-OH complex **2** underwent demetalation to the extent of 23% in 3 h (see Supporting Information, Table S1). The fact that **3** did not undergo demetalation was gratifying since a stable Al^{18/19}F chelate is our most important prerequisite. It was interesting to note that fluoride, which is bioisosteric with both the hydrogen atom and the hydroxyl group, could displace the hydroxide from **2** (Figure 3E). The driving force for this reaction may be the formation of the strong Al-F bond (580 – 670 kJ/mol) in the product.

We believe that the improved labeling efficiency with peptides covalently linked to NODA-MPAA is due to the presence of a N₃O₂ donor set that can form stable mononuclear octahedral complexes with aluminum and possess a single coordination site for binding fluorine. The N₃O₃ donor set (hexadentate) of TACN derivatives like NOTA provide a suitable environment for the formation of highly stable aluminum chelates.²³ Low RCYs with IMP449 (N₃O₃ donor set) may result from the coordination sphere of aluminum being saturated, with limited access for fluorine. A similar situation, although with less restriction, could occur with IMP461, due to the participation of the amido oxygen in the complexation. Under identical conditions, RCYs were higher with IMP485, when compared to IMP449 and IMP461 (see Supporting Information, Tables S3–S5). These results suggest that pentadentate ligands with a free site for binding fluorine are ideal for stable Al¹⁸F chelation.

The ¹⁸F[−] produced in the cyclotron comes out as a dilute solution in H₂18O and is generally contaminated with metals from the target cell. Since our ¹⁸F-labeling method involves the use of a BFC, the presence of competing metal ions would hamper effective labeling.³³ Initial labeling studies were performed with ¹⁸F[−] that was bound onto a (anion exchange resin) QMA cartridge, eluted with 0.4 M KHCO₃, followed by neutralization of the KHCO₃ with acetic acid. With pH being an important parameter for radiofluorination, inconsistencies in the neutralization resulted in varying RCYs. Recently, we found that QMA-bound ¹⁸F[−] could be eluted with 0.9% saline and used without making any pH adjustments.²⁰ Subsequently, we obtained high RCYs with the commercially available USP grade Na¹⁸F, an approved bone-imaging agent.³⁴ This was convenient, since it eliminated the concentration, purification and dry-down steps associated with other ¹⁸F-labeling methods.

We also demonstrate herein two routes to synthesize the ^{18}F -labeled peptide, either heat the hapten-peptide (IMP485) **4** and Al^{3+} with commercially available $^{18}\text{F}^-$ in saline or synthesize the peptide-aluminum complex **5** and react it with Na^{18}F (see Supporting Information, Scheme S1). The latter approach is attractive since it is based on the reaction of a single reactant with Na^{18}F , eliminating any speculation regarding peptide to Al^{3+} ratios or inadvertently not adding any Al^{3+} . One of the key findings during the development of this methodology was that the presence of an added hydrophilic organic co-solvent (generally 50% or more v/v) doubled the RCYs (Table 2). However, ethanol was the co-solvent of choice for most of our subsequent radiofluorinations, since it is biocompatible and has the added advantage of preventing radiolysis.³⁵ Based on these findings, we explored the possibility of developing a single-vial kit, requiring only the addition of $^{18}\text{F}^-$ in saline.³⁶ These studies included an examination of the effects of temperature, reaction time, pH, amount of peptide, peptide to Al^{3+} ratio, and reaction volume on the radiolabeling efficiency. The development of the lyophilized kit formulation of IMP485, as well as kits for NODA-MPAA derived somatostatin receptor and bombesin binding peptides, will be reported elsewhere.

The theoretical specific activity (SA) of carrier-free $^{18}\text{F}^-$ is 1702 Ci/ μmol , but varies from 8.5 to 1162 Ci/ μmol , due to isotopic dilution from water, reagents and reaction vessels.³⁷ In most clinical applications, 5 to 10 mCi of the ^{18}F -labeled product with specific activities >1 Ci/ μmol are required for imaging cellular targets.³⁸ We recently reported the development of a lyophilized kit formulation containing 20 nmol IMP485 that has been radiolabeled to a specific activity of ~ 4 Ci/ μmol .³⁶ This would allow for 4 h from start of radiolabeling to the injection of the patient to retain a SA of 1 Ci/ μmol . When the reaction mixture is purified by SPE, one has to bear in mind that the product is a mixture consisting of unlabeled material (IMP485) **4** plus AlOH(IMP485) **5**, with $\text{Al}^{18/19}\text{F}$ -labeled product. As seen in the Supporting Information Figures S4 and S5, excellent separation of $\text{Al}^{19}\text{F(IMP485)}$ **6** from (IMP485) **4** and AlOH(IMP485) **5** using a C_{18} column is possible. Therefore, the SA could be increased if the post-labeling purification was by RP-HPLC instead of SPE. However, as mentioned earlier, the precise SA of the ^{18}F -labeled product will also depend on the SA of the $^{18}\text{F}^-$.³⁷ Due to the short half-life of ^{18}F , labeling is frequently performed with high levels of radioactivity, and this in turn imposes a requirement that the radiochemistry be performed in an apparatus that is shielded from the operator and is fully automated.³⁹ Electrochemical methods, currently being explored for the concentration of no-carrier-added $^{18}\text{F}^-$ will help in reducing the reaction volume.⁴⁰ We are confident that ^{18}F -labeling via Al^{18}F chelation, particularly using single-vial kits, can be adapted for use in these automated systems, and would also benefit from microfluidic technology.⁴¹

Advancement of an ^{18}F -labeled peptide into the clinic requires that it possess good *in vivo* stability, excellent targeting, and favorable excretion profile. Animal studies performed using kit formulated IMP485 have shown rapid clearance from the body via the kidneys with negligible presence in the bone, supporting *in vivo* stability.⁴² To evaluate the true potential of this novel BFC **1**, we have synthesized and ^{18}F -labeled somatostatin receptor-binding and bombesin peptides, and are currently investigating their tumor targeting capabilities.

Finally, while we appreciate that ^{18}F -labeling via Al^{18}F chelation is facile, it has only been described for peptides that are stable at high temperatures. Recently, we reported a maleimide derivative of NODA-MPAA that can be ^{18}F -labeled and then coupled with high efficiency to thiol-containing peptides and proteins.⁴³ This two-step approach opens the possibility for adapting the Al^{18}F chelation to heat-sensitive biomolecules.

CONCLUSIONS

A new pentadentate BFC **1** was synthesized and reacted with AlF_3 to yield an organofluoroaluminate **3**, which was characterized by RP-HPLC, ^1H and ^{13}C NMR, HRMS, and X-ray crystallography. The solid-state X-ray crystal structure of **3** reveals aluminum coordinated by an N_3O_2 donor set, in slightly distorted octahedron geometry, with fluorine occupying one of the axial positions. The stability of **3** was confirmed when it showed no signs of demetalation over 24 h, when incubated in PBS, pH 7.4, at 37 °C. The partial solubility of AlF_3 led us the realization that the addition of a hydrophilic organic co-solvent improves the yield of the organofluoroaluminate. It is interesting to note that the reaction of **1** with AlCl_3 and AlF_3 yields two distinct chelates, (Al-OH) **2** and (AlF) **3**, respectively. High yield aqueous radiofluorination was accomplished in a single step, by heating a mixture of hapten-peptide **4** and Al^{3+} or the peptide-aluminum complex **5**, with commercially available $^{18}\text{F}^-$ in saline. The post-labeling purification method (SPE or RP-HPLC) employed was dictated by specific activity requirements. We hope that this robust, user-friendly ^{18}F -labeling methodology will stimulate the rapid development of new PET tracers for the imaging of disease states.

MATERIALS AND METHODS

General Information

All commercially obtained chemicals were analytical grade and used without further purification. $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, NaF, $\text{AlF}_3 \cdot 3\text{H}_2\text{O}$, and 4-(bromomethyl)-phenylacetic acid were purchased from Sigma-Aldrich (Milwaukee, WI) and sodium acetate and acetic acid from J. T. Baker (Phillipsburg, NJ). NO_2ArBu was purchased from CheMatech (Dijon, France).

Fmoc protected amino acids, seiber amide resin, trifluoroacetic acid were obtained from Creosalus (Louisville, KY). The peptides were synthesized using Protein Technologies, Inc. (Tucson, AZ) peptide synthesizer. The analytical and preparative reverse-phase HPLC (RP-HPLC) columns were purchased from Phenomenex (Torrance, CA) and Waters Corp. (Milford, MA). Two millimolar solutions of AlCl_3 , NaF, AlF_3 , and **1** were prepared in 2 mM NaOAc, the pH being maintained in the range of 3.8 to 4.4. Since **1** and **4** displayed a high propensity for complexation with other metals (e.g., copper, gallium and indium) at room temperature, care was taken to ensure that reactants were prepared in metal free containers. Reactions were performed in sealed 2-mL microcentrifuge tubes with cap and O-ring obtained from Fisher Scientific (Agawan, MA). Radiolabeled peptides were purified using Waters Oasis 1 cm^3 or 3 cm^3 flangeless cartridges. $^{18}\text{F}^-$ in saline was purchased from PETNET (Hackensack, NJ). Solid-phase extraction (SPE) cartridges (Sep-Pak light QMA, Sep-Pak Accell plus CM, and Oasis HLB) were obtained from Waters (Milford, MA). ^1H and ^{13}C NMR spectra were recorded using Varian Inova NMR spectrometer (Varian, Inc., Palo Alto, CA) at 500 MHz for ^1H and 125.7 MHz for ^{13}C at Rutgers University (Newark, NJ). ^1H NMR spectra are referenced to the tetramethylsilane peak ($\delta = 0$). Product identification was verified by high-resolution mass spectroscopy (HRMS) using positive mode electrospray ionization with an Agilent Time-of-Flight LC-MS at Immunomedics, Inc. (Morris Plains, NJ). X-ray crystallography data were collected on Bruker-Nonius KappaCCD (Mo $K\alpha$ radiation) diffractometer at Hunter College of the City University of New York (New York, NY). All calculations were performed using Bruker SHELXS-97 software package, while SHELXL-97 was used for refinement.⁴⁴

HPLC Methods

Analytical HPLC was performed using a Waters 2695 system equipped with a Phenomenex Gemini C_{18} reverse-phase column (250 $\times$ 4.6 mm, 5 μm , 110 Å).

Method 1—Gradient of 1 min with 100% A (0.1% TFA), then to 90:10 A/B over 5 min, followed by 85:15 A/B (90% acetonitrile, 10% water, 0.1% TFA) over 30 min at a flow rate of 1 mL/min, absorbance was detected at 220 and 254 nm using Waters 2996 photodiode array (PDA) detector.

Method 2—Gradient of 1 min with 100% A (0.1% TFA), then to 90:10 A/B over 5 min, followed by 87:13 A/B (90% acetonitrile, 10% water, 0.1% TFA) over 20 min at a flow rate of 1 mL/min, absorbance was detected at 220 and 254 nm using Waters 2996 photodiode array (PDA) detector. The column effluent was monitored using Perkin Elmer 610TR Radiomatic Flow scintillation analyzer.

Method 3—Linear gradient of 100% C (H₂O) to 30% D (50% ethanol) over 20 min at a flow rate of 1 mL/min, absorbance was detected at 220 and 254 nm using Waters 2996 photodiode array (PDA) detector.

Method 4—Products were purified using Waters PrepLC 4000 system with Sunfire Prep C₁₈ OBD reverse-phase column (150 × 30 mm, 5 μm) using a linear gradient of 100% A (0.1% TFA) to 15% B (90% acetonitrile, 10% water, 0.1% TFA) over 80 minutes at a flow rate of 45 mL/min, absorbance was detected at 220 nm using Waters 486 tunable absorbance detector.

Method 5—Product was purified using Waters PrepLC 4000 system with Sunfire Prep C₁₈ OBD reverse-phase column (150 × 30 mm, 5 μm) using a linear gradient of 100% A (0.006 N HCl) to 100% B (acetonitrile) over 60 minutes at a flow rate of 45 mL/min, absorbance was detected at 220 nm using Waters 486 tunable absorbance detector.

Synthesis of the BFCs

2-(4-(carboxymethyl)-7-[[4-(carboxymethyl)phenyl]methyl]-1,4,7-triazacyclononan-1-yl)acetic acid [H₂L] (1). (NODA-MPAA)—To a solution of 4-(bromomethyl)phenylacetic acid (15.7 mg, 0.68 mmol) in anhydrous CH₃CN at 0 °C was added dropwise over 20 min a solution of NO₂AtBu (26 mg, 0.73 mmol) in CH₃CN (5 mL). After 2 h, anhydrous K₂CO₃ (5 mg) was added to the reaction mixture and allowed to stir at room temperature overnight. Solvent was evaporated and the concentrate was acidified with 2 mL TFA. After 3 h, the reaction mixture was diluted with water and purified by preparative RP-HPLC (Method 4) to yield a white solid (11.8 mg, 43.7%). ¹H NMR (500 MHz, DMSO-d₆, 25 °C) δ 2.65–3.13 (m, 12 H), 3.32 (d, 2H), 3.47 (d, 2H), 3.61 (s, 2 H), 4.32 (s, 2 H), 7.33 (d, 2H), 7.46 (d, 2H); ¹³C (125.7 MHz, DMSO-d₆) 40.8, 47.2, 49.6, 50.7, 55.2, 58.1, 130.4, 130.5, 130.9, 136.6, 158.4, 158.7, 172.8, 172.9. HRMS (ESI) calculated for C₁₉H₂₇N₃O₆ (M+H)⁺ 394.1973; found 394.1979.

2-{4-[(4,7-bis-*tert*-butoxycarbonylmethyl)-[1,4,7]-triazacyclononan-1-yl)methyl]phenyl}acetic acid (7)—To a solution of 4-(bromomethyl)phenylacetic acid (593 mg, 2.59 mmol) in anhydrous CH₃CN (50 mL) at 0 °C were added dropwise over 1 h a solution of NO₂AtBu (1008 mg, 2.82 mmol) in CH₃CN (50 mL). After 4 h, anhydrous K₂CO₃ (100.8 mg, 0.729 mmol) was added to the reaction mixture and allowed to stir at room temperature overnight. Solvent was evaporated and the crude was purified by preparative RP-HPLC (Method 5) to yield a white solid (713 mg, 54.5%). ¹H NMR (500 MHz, CDCl₃, 25 °C, TMS) δ 1.45 (s, 18 H), 2.64–3.13 (m, 16 H), 3.67 (s, 2 H), 4.38 (s, 2 H), 7.31 (d, 2H), 7.46 (d, 2H); ¹³C (125.7 MHz, CDCl₃) δ 28.1, 41.0, 48.4, 50.9, 51.5, 57.0, 59.6, 82.3, 129.0, 130.4, 130.9, 136.8, 170.1, 173.3. HRMS (ESI) calculated for C₂₇H₄₃N₃O₆ (M+H)⁺ 506.3225, found 506.3210.

Synthesis of Aluminum chelates

AIOH(NODA-MPAA) (2)—H₂L **1** (19.8 mg, 0.05 mmol) was dissolved in 1 mL of 2 mM NaOAc, pH 4.4 and treated with (12 mg, 0.05 mmol) AlCl₃·6H₂O. The pH was adjusted to 4.5–5.0 and the reaction mixture was refluxed for 15 min. The crude was purified by preparative RP-HPLC (Method 4) to yield a white solid (10.9 mg, 49.8%). HRMS (ESI) calculated for C₁₉H₂₆AlN₃O₇ (M+H)⁺ 436.1659; found 436.1664.

AlF(NODA-MPAA) (3)—H₂L **1** (40.6 mg, 0.103 mmol) was dissolved in 1 mL of 2 mM NaOAc, pH 4.4, 0.5 mL ethanol and treated with (19.5 mg, 0.141 mmol) AlF₃·3H₂O. The pH was adjusted to 4.5–5.0 and the reaction mixture was refluxed for 15 min. On cooling, the pH was once again raised to 4.5–5.0 and the reaction mixture refluxed for 15 min. The crude was purified by preparative RP-HPLC (Method 4) to yield a white solid (22.4 mg, 49.6%). ¹H NMR (500 MHz, DMSO-d₆, 25 °C) δ 2.24–3.51 (m, 16 H), 3.59 (s, 2 H), 3.74 (d, 1H), 4.24 (d, 1H), 7.29 (d, 2H), 7.40 (d, 2H); ¹³C (125.7 MHz, DMSO-d₆) 40.7, 46.0, 51.9, 52.7, 53.0, 54.5, 59.6, 64.0, 129.9, 131.3, 132.5, 135.8, 172.0, 172.2, 172.9. HRMS (ESI) calculated for C₁₉H₂₅AlFN₃O₆ (M+H)⁺ 438.1616; found 438.1627.

Synthesis of the Peptide and its Aluminum chelates

IMP485 (4)—NODA-MPAA-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂. The peptide was synthesized on Sieber amide resin with the amino acids added in the following order: Aloc-D-Lys(Fmoc)-OH, Trt-HSG-OH, Aloc removal, Fmoc-D-Tyr(But)-OH, Aloc-D Lys(Fmoc)-OH, Trt-HSG-OH, Aloc removal, and (tBu)₂NODA-MPAA. The peptide was then cleaved and purified by preparative RP-HPLC (Method 4). HRMS (ESI) calculated for C₆₂H₈₉N₁₇O₁₅ (M+H)⁺ 1312.6797; found 1312.6815.

AIOH(IMP485) (5)—To IMP485 (**3**) (21.5 mg, 0.016 mmol) was dissolved in 1 mL of 2 mM NaOAc, pH 4.4 and treated with (13.2 mg, 0.055 mmol) AlCl₃·6H₂O. The pH was adjusted to 4.5–5.0 and the reaction mixture was refluxed for 15 min. The crude was purified by preparative RP-HPLC (Method 4) to yield a white solid (11.8 mg). HRMS (ESI) calculated for C₆₂H₈₈AlN₁₇O₁₆ (M+H)⁺ 1354.6483; found 1354.6431.

Al¹⁹F(IMP485) (6)—To IMP485 (**3**) (16.5 mg, 0.013 mmol) was dissolved in 1 mL of 2 mM NaOAc, pH 4.43, 0.5 mL ethanol and treated with (2.5 mg, 0.018 mmol) AlF₃·3H₂O. The pH was adjusted to 4.5–5.0 and the reaction mixture was refluxed for 15 min. On cooling the pH was once again raised to 4.5–5.0 and the reaction mixture refluxed for another 15 min. The crude was purified by preparative RP-HPLC (Method 4) to yield a white solid (10.3 mg). HRMS (ESI) calculated for C₆₂H₈₇AlFN₁₇O₁₅ (M+H)⁺ 1356.6440; found 1356.6458.

¹⁸F-labeling of IMP485—10 μL (20 nmol) IMP485, 5 μL AlCl₃ (10 nmol), 100 μL Na¹⁸F in 0.9% saline (34.6 mCi), and 110 μL ethanol were reacted in 2 mL microcentrifuge tube (sealed) at 105 °C for 15 min. The reaction mixture was cooled, diluted with 2–3 mL deionized water and then transferred into a HLB 3 cm³ cartridge. The solution was eluted under vacuum into an empty 10 mL crimp-sealed vial. The reaction vessel and column were rinsed with 3 × 1 mL portions of water. The HLB cartridge was then transferred to another empty 3 mL vial and the product eluted with 4 × 150 μL 1:1 ethanol/water to yield 20.3 mCi of Al¹⁸F(IMP485) (decay corrected RCY 74.1%; SA 1.01 Ci/μmol).

¹⁸F-labeling of AIOH(IMP485)—10 μL (20 nmol) AIOH(IMP485), 100 μL Na¹⁸F in 0.9% saline (43.3 mCi), and 110 μL ethanol were reacted in 2 mL microcentrifuge tube (sealed) at 105 °C for 15 min. The reaction mixture was cooled, diluted with 2–3 mL deionized water and then transferred into a HLB 3 cm³ cartridge. The solution was eluted

under vacuum into an empty 10 mL crimp-sealed vial. The reaction vessel and column were rinsed with 3×1 mL portions of water. The HLB cartridge was then transferred to another empty 3 mL vial and the product eluted with 4×150 μ L 1:1 ethanol/water to yield 25.6 mCi of $\text{Al}^{18}\text{F}(\text{IMP485})$ (decay corrected RCY 72.3%; SA 1.28 Ci/ μ mol).

Radio-HPLC

The crude reaction mixture containing unbound $^{18}\text{F}^-$ and the product obtained after purification by SPE were analyzed by RP-HPLC (Method 2) using a Perkin Elmer 610TR Radiomatic Flow scintillation analyzer (see Supporting Information, Figure S2), with recoveries of 79.7% and 100.6%, respectively.

Serum Stability

The SPE purified $\text{Al}^{18}\text{F}(\text{IMP485})$ in 20 μ L 1:1 EtOH/ H_2O (66.3 μ Ci) was mixed with 200 μ L of human serum, placed in the HPLC autosampler heated to 37 $^\circ\text{C}$ and injected 1, 2 and 4 μ L at time 0, 2 and 4 h, respectively, to account for physical decay (see Supporting Information, Figure S3). No detectable $^{18}\text{F}^-$ above background at the void volume was observed up to 4 h. In addition, percent recovery for each run was determined by counting the activity in 6 fractions over the entire 20 min run against a standard prepared from the original mixture. Total recoveries ranged from ~76% to 91%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of IMP449 and IMP461.

Figure 2.
Structures of NODA-MPAA, IMP485, and their aluminum complexes.

Figure 3. HPLC chromatograms using Method 1: (A) AlCl_3 with **1**; (B) AlCl_3 + NaF with **1**; (C) AlF_3 with **1**; (D) AlF_3 with **1** in the presence of MeOH; (E) NaF with **2**; (F) NaF with **2** in the presence of EtOH.

Figure 4.
 ^1H NMR of **1** and **3** in DMSO-d_6 at room temperature (500 MHz).

Figure 5.
X-ray crystal structure of $\text{Al}^{19}\text{F}(\text{NODA-MPAA}) \mathbf{3}$

Figure 6. HPLC chromatograms (A) SPE-purified $\text{Al}^{18}\text{F}(\text{IMP485})$. (B) $\text{Al}^{19}\text{F}(\text{IMP485})$.

Scheme 1.
Reaction of **1** with AlCl_3 and AlF_3 .

Scheme 2.
Synthesis of NODA-MPAA **1** and $(t\text{Bu})_2\text{NODA-MPAA}$ **7**.

Table 1¹⁸F-labeling of 20 nmol IMP485 + 10 nmol Al³⁺ with varying amounts of Na¹⁸F

Activity ^a Na ¹⁸ F(mCi)	Aqueous(μL)	Ethanol(μL)	Isolated activity ^b (mCi)	RCY ^c (%)
1.02	50	0	0.21	23.9
0.92	50	50	0.60	79.9
3.20	50	50	2.35	89.3
6.20	50	50	4.42	86.7
12.34	100	100	8.35	84.4
34.60	115	110	20.3	74.1 ^d

^a 10 μL IMP485, 5 μL Al³⁺, 105–110 °C, 15 min.^b Isolated activity in (1:1) EtOH/H₂O after HLB column purification (SPE).^c decay corrected RCY – based on synthesis time of 31 – 37 minutes.^d Specific activity: 1.01 Ci/μmol.

Table 2Effect of solvent on the ^{18}F -labeling of IMP485

Activity ^a Na ¹⁸ F(mCi)	Solvent(50 μL)	Isolated activity ^b (mCi)	RCY ^c
1.416	–	0.516	43.5
1.326	EtOH	1.006	89.4
1.206	DMF	0.886	86.5
1.608	DMSO	1.152	86.0
1.246	CH ₃ CN	0.966	91.3

^a 35–50 μL Na F, 10 μL IMP485, 5 μL Al, 105 C, 15 min.^b Isolated activity in (1:1) EtOH/H₂O after HLB column purification (SPE).^c decay corrected RCY – based on synthesis time of 26 – 29 minutes.

Table 3¹⁸F-labeling of 20 nmol AlOH(IMP485) with varying amounts of Na¹⁸F

Activity ^a Na ¹⁸ F(mCi)	Aqueous(μL)	Ethanol(μL)	Isolated activity ^b (mCi)	RCY ^c (%)
3.51	70	–	1.019	36.4
3.34	70	100	2.21	83.6
4.07	60	75	2.44	73.8
43.3	110	110	25.6	72.3 ^d

^a 10 μL AlOH(IMP485), 105 °C, 15 min.^b Isolated activity in (1:1) EtOH/H₂O after HLB column purification (SPE).^c decay corrected RCY – based on synthesis time of 32 – 37 minutes.^d Specific activity: 1.28 Ci/μmol.

Table 4Bond lengths (Å) and bond angles (°) for complex **3**

Bond lengths (Å)		Bond angles (°)	
Al-F	1.714 (1)	F-Al-N1	176.2 (1)
Al-O12	1.854 (2)	F-Al-N4	96.8 (1)
Al-O14	1.875 (2)	F-Al-N7	91.8 (1)
Al-N1	2.074 (2)	F-Al-O12	97.7 (1)
Al-N4	2.036 (2)	F-Al-O14	93.3 (1)
Al-N7	2.080 (2)	O12-Al-O14	95.6 (1)
N1-C2	1.487 (3)	O12-Al-N1	82.6 (1)
N1-C9	1.506 (3)	O12-Al-N4	165.4 (1)
N1-C10	1.482 (3)	O12-Al-N7	95.3 (1)
N4-C3	1.505 (3)	O14-Al-N1	90.4 (1)
N4-C5	1.492 (3)	O14-Al-N4	83.3 (1)
N4-C12	1.483 (3)	O14-Al-N7	167.2 (1)
N7-C6	1.504 (3)	N1-Al-N4	82.9 (1)
N7-C8	1.496 (3)	N1-Al-N7	84.5 (1)
N7-C14	1.594 (2)	N4-Al-N7	84.4 (1)