The Design and Synthesis of Highly Branched and Spherically Symmetric Fluorinated Macrocyclic Chelators

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Abstract

Two novel, highly fluorinated macrocyclic chelators with highly branched and spherically symmetric fluorocarbon moieties have been designed and efficiently synthesized. This is achieved by conjugating a spherically symmetric fluorocarbon moiety to the macrocyclic chelator DOTA, with or without a flexible oligo-oxyethylene linker between these two parts. As a result of the spherical symmetry, all 27 fluorine atoms in each fluorinated chelator give a sharp singlet ^{19}F NMR signal. The hydrophilicity and the ^{19}F relaxation behavior of fluorinated chelators can be modulated by the insertion of a flexible linker between the fluorocarbon moiety and the macrocyclic linker. These chelators serve as prototypes for ^{1}H-^{19}F dual-nuclei magnetic resonance imaging agents.

Keywords

fluorinated macrocyclic chelator; fluorinated amphile; ^{19}F NMR; spherical symmetry; DOTA

We are interested in using fluorocarbon liquid nanoparticles, formulated as microemulsions, as multifunctional drug delivery vehicles for ^{19}F MR image-guided targeted drug therapy, particularly for radionuclide therapy.[1] In our first paper of this series, the synthesis of prototypical fluorinated oils (*F*-oils) and fluorinated amphiles (*F*amphiles) was described.[2] *F*-oils and *F*-amphiles (serving as emulsifiers) will constitute the inner core and the outer shell of the fluorocarbon nanoparticles, respectively. To facilitate ^{19}F MR imaging, the fluorocarbon moiety, containing three perfluoro-*tert*-butyl groups centered around a pentaerythritol hub, is designed to be highly branched and spherically symmetric so that all fluorine atoms will give a sharp singlet ^{19}F signal. To enable the nanoparticles with ^{1}H-^{19}F dual nuclei MR imaging capacity, some highly fluorinated chelators are needed. Detailed discussions of the design principles of the nanoparticle (e.g., rationale for ^{1}H-^{19}F dual nuclei MR imaging) and that of *F*-oils and *F*-amphiles (e.g., the rationale for size and shape matching and branching) can be found in references [1] and 2, respectively.

In this paper, the syntheses of two highly fluorinated macrocyclic chelators are described. For targeted radionuclide therapy, a macrocyclic chelator serves two functions: carrying metallic radionuclides (e.g., $^{90}Y_{3+}$) for radiotherapy and carrying Gd_{3+} for contrast-enhanced ^{1}H MR imaging (See Figure 1 of reference 1). The macro-cyclic chelator is conjugated to a fluorocarbon moiety so that it can be incorporated into the outer shell of a fluorocarbon

nanoparticle. Note that a fluorinated chelator is an *F*-amphile as the fluorocarbon moiety is hydrophobic while the chelator is hydrophilic.

We choose the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetate) and its derivative for our application because DOTA forms very stable complexes with metallic ions,[3] which is essential for radionuclide therapy. DOTA and its derivative are already in clinical use as MR imaging contrast agents (Dotarem® and ProHance®). DOTA is also the chelator used in the radiotherapeutic drug candidate (OctreoTher®), which is under clinical trial.[4] Hence, DOTA has a proven record in radionuclide therapy and MR imaging.

In its simplest form, DOTA is conjugated directly to the fluorocarbon moiety, forming the chelator *F*-DOTA **1**, as shown in Figure 1. However, it might be necessary to insert a flexible hydrophilic linker between the macro-cyclic chelator and the fluorocarbon moiety to increase amphipathicity and to alleviate potential steric hindrance to chelation caused by the bulky fluorocarbon moiety. An obvious choice for the flexible linker is oligo-oxyethylene as oxyethylene is hydrophilic and biocompatible. One such chelator with a tetra-oxyethylene linker (*F*-DOTA **2**) is presented in Figure 1. The fluorocarbon moieties of both molecules have spherical symmetry.

The purpose of this synthesis paper is to establish the feasibility of conjugating a bulky fluorocarbon moiety to the macrocyclic DOTA and the feasibility of inserting an oligo-oxyethylene linker between the fluorocarbon moiety and DOTA. Detailed investigation of metal ion chelation and MR imaging capacity will be conducted in the future.

The synthesis of *F*-DOTA **1** is depicted in Scheme 1. By employing the method described in our previous paper,[2] the highly fluorinated alcohol **3** was prepared on a 50-gram scale that will be used as the common starting material for both *F*-DOTA **1** and *F*-DOTA **2** synthesis. Treatment of alcohol **3** with trifluoromethanesulfonic anhydride in the presence of pyridine gave triflate **4** with excellent yield, which was isolated through phase separation after the addition of 10% water (v/v) to the reaction mixture. It is noteworthy that tetrahydrofuran, instead of the commonly used dichloromethane for such esterification, turned out to be the ideal solvent for this reaction because alcohol **3** has very limited solubility in dichloromethane. It is necessary to use excess of trifluoromethanesulfonic anhydride and pyridine to achieve high conversion of the well buried highly fluorinated alcohol **3** to triflate **4**. Triflate **4** was then conjugated to the cyclen ring after reacting with 2 equivalents of cyclen in a mixture of tetrahydrofuran and dichloromethane (1/1, v/v) at 45 °C overnight. The product **5** was extracted by FC-72 (perfluorohexanes) from the dried reaction residue (dissolved in dichloromethane) with an 88% yield. Again, the choice of solvent is crucial for the conjugation. Instead of reacting with dissolved cyclen, triflate **4** decomposed slowly, when dichloromethane or chloroform alone was used as the solvent, because it is not stable at ambient temperature and has low solubility in dichloromethane or chloroform. The reaction of compound **5** with ethyl bromoacetate in the presence of potassium carbonate in a mixture of tetrahydrofuran/ dimethylformamide (1/1, v/v) at 60 °C provided ester **6** with an excellent yield after flash chromatography purification on neutral aluminum oxide. Finally, hydrolysis of the three ethyl esters in **6** with lithium hydroxide in water and methanol afforded the target molecular *F*DOTA **1** with a 97% yield.

Before starting the synthesis of the target molecule *F*-DOTA **2**, the tetra-oxyethylene linker **9** between the fluorocarbon moiety and the DOTA moiety was synthesized with good yield on a 50-gram scale from commercially available tetraethylene glycol **7** by selectively protecting one of the hydroxyl groups with benzyl bromide[5] and transforming the other hydroxyl group into mesylate (Scheme 2). It is an improvement to our former synthesis[2a] by changing the protective group for the hydroxyl group in tetraethylene glycol **7** from the *tert-*

butyldimethylsilyl (TBDMS) group to the benzyl (Bn) group because the mesylate **9** can be prepared on a larger scale and in a much simpler and cheaper way. This also reduces side reaction (when coupling **9** with alcohol **3** in Scheme 3) and simplifies the purification process (when deprotecting **10** in Scheme 3) for subsequent steps.

With **9** in hand, the synthesis of *F*-DOTA **2** proceeded (Scheme 3). The highly fluorinated alcohol **3** was first attached to the hydrophilic tetra-oxyethylene linker **9** to afford compound **10** with good yield. Then, removal of the benzyl group in compound **10** by palladium hydroxide catalyzed hyodrogenolysis gave alcohol **11** with an excellent yield which was then treated with methanesulfonyl chloride and triethyl amine to give the mesylate **12** with a quantitative yield. Attaching cyclen to the fluorocarbon moiety was achieved by treating compound **12** with 2 equivalents of cyclen. However, the resulting cyclen derivative **13** can hardly be isolated from the reaction mixture by flash chromatography. Fortunately, solid-phase extraction of the reaction mixture on fluorinated silica gel[6] provided amine **13** with a 93% yield. Compound **13** was then reacted with ethyl bromoacetate to afford ester **14** with a good yield. Finally, treatment of compound **14** with lithium hydroxide gave the target molecule *F*-DOTA **2** with a 99% yield.

With *F*-DOTA **1** and *F*-DOTA **2** in hand, the ^{19}F NMR spectrum of each chelator was then acquired. As designed, both *F*-DOTA **1** and *F*-DOTA **2** gave only one sharp singlet ^{19}F NMR signal with a peak width of around 0.02 ppm (Figure 2). These highly fluorinated chelators are ideal for ^{19}F MR imaging applications because a sharp singlet ^{19}F NMR signal can not only eliminate chemical shift artifacts but also get rid of the signal intensity modulation caused by J-coupling from adjacent ^{19}F or ^{1}H atoms.[7]

The flexible oligo-oxyethylene linker is the variable portion of our chelator design. Hence, its impact on the physicochemical properties of fluorinated chelators is assessed. The impact of the tetra-oxyethylene linker on the hydrophilicity of the chelator was evaluated by comparing the 1-octanol/water partition coefficients (P_{oct}) of *F*-DOTA **1** and *F*-DOTA **2**. P_{oct} is a standard physicochemical parameter in assessing the hydrophilicity of pharmaceuticals.[8] P_{oct} of *F*-DOTA **1** and *F*-DOTA **2** was determined by ^{19}F NMR.[9] P_{oct} of *F*-DOTA **1** and *F*-DOTA **2** are 7.63 and 1.40×10^{-2}, respectively. Hence, the tetra-oxyethylene linker significantly increased the hydrophilicity of the fluorinated chelator.

The NMR signal relaxation times, T_1 and T_2 are important parameters for MR imaging because MRI contrasts are T_1- or T_2- weighted. The impact of the tetra-oxyethylene linker on ^{19}F relaxation behavior was evaluated by NMR spectroscopy (376 MHz). *F*-DOTA **1** and *F*-DOTA **2** in methanol-*d4* at a concentration of 0.025 M gave the following T_1 and T_2 values: *F*-DOTA **1**, T_1 = 708 ms, T_2 = 424 ms; *F*-DOTA **2**, T_1 = 1067 ms, T_2 = 824 ms (As a reference point, the trifluoromethyl group of perfluorooctyl bromide, a fluorocarbon compound previously used in ^{19}F MRI, has a T_1 of 2881 ms and a T_2 of 2184 ms in methanol-*d4* under the same condition[10]). This demonstrates clearly that, in addition to modulating hydrophilicity, the oligo-oxyethylene linker can also modulate T_1 and T_2 of fluorinated chelators.

In short, both the hydrophilicity and the ^{19}F relaxation behavior of fluorinated chelators can be modulated by the addition of the flexible linker. Hence, one way to improve the physicochemical properties of future generation of fluorinated chelators for MR imaging applications is to engineer the flexible linker to achieve desired aqueous solubility and ^{19}F relaxation profile.

Experimental Section

^{1}H NMR spectra were recorded with trimethylsilane as internal reference and ^{19}F NMR spectra were recorded with hexafluorobenzene as internal reference on a Varian 400 spectrometer.

Measurements of pH in mobile phases for high performance liquid chromatography (HPLC) were taken after pH calibration with aqueous standard solutions. Molecular mass was obtained using a Voyager-DE MALDI-TOF mass spectrometer.

Triflate 4

To a stirred solution of alcohol **3** (7.9 g, 10.0 mmol) and pyridine (8.2 mL, 7.9 g, 100.0 mmol) in tetra-hydrofuran (250 mL) was added dropwise a solution of trifluoromethanesulfonic anhydride (8.2 mL, 14.1 g, 50.0 mmol) in tetrahydrofuran (20 mL) at 0 °C. After stirring at this temperature for 1 h, the reaction was quenched by slow addition of water (27 mL). The mixture was then transferred to a separatory funnel and the lower phase was collected. Washing the oil with dichloromethane gave the pure triflate **4** as a clear oil (8.8 g, 96% yield) (This compound is unstable at rt. and needs to be used immediately after preparation). ^{1}H NMR (400 MHz, Acetone-*d6*) δ 4.82 (s, 2H), 4.39 (s, 6H); ^{19}F NMR (376 MHz, Acetone-*d6*) δ 71.21 (s, 27F), 75.93 (s, 3F).

Amine 5

A suspension of triflate **4** (8.5 g, 9.3 mmol) and cyclen (3.3 g, 18.9 mmol) in a mixture of tetrahydrofuran and dichloromethane (50 mL/50 mL) was stirred at rt. for 2 h. Then the reaction temperature was slowly raised to 45 °C and the mixture was then stirred at this temperature overnight. After concentrating the reaction mixture to dryness under vacuum, the residue was dissolved in dichloromethane (50 mL) and extracted with FC-72 (50 mL, three times). Evaporation of the combined FC-72 phase gave the product **5** as a clear oil (7.7 g, 88% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.19 (s, 6H), 2.72–2.75 (m, 6H), 2.58–2.60 (m, 4H), 2.51–2.54 (m, 8H); ^{19}F NMR (376 MHz, CDCl$_3$) δ −73.31 (s); ^{13}C NMR (100.7 MHz, CDCl$_3$) δ 120.1 (q, $J = 293.3$ Hz), 79.1–80.0 (m), 68.4, 54.4, 53.4, 46.9, 46.0, 45.7, 45.5; MS (CI) m/z 945 ((M+H)$^+$, 100); HRMS (CI) calcd for C$_{25}$H$_{28}$F$_{27}$N$_4$O$_3$ 945.1730, found 945.1717.

Tri-ethyl ester 6

Powdered potassium carbonate (8.3 g, 60.2 mmol) and ethyl bromoacetate (4.2 mL, 6.3 g, 37.5 mmol) were added to a stirred solution of amine **5** (7.1 g, 7.5 mmol) in a mixture of tetrahydrofuran and dimethyl-formamide (35 mL/ 35 mL) at rt. and the resulting suspension was stirred at 60 °C overnight. After filtration, the solvent was removed under vacuum and the residue was purified by flash chromatography on neutral aluminum oxide (CH$_2$Cl$_2$/Methanol = 10/1) to give the product **6** as a clear oil (6.3 g, 92% yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.03–4.10 (m, 12H), 3.34 (s, 2H), 3.25 (s, 4H), 2.62–2.76 (m, 18H), 1.16–1.21 (m, 9H); ^{19}F NMR (376 MHz, CDCl$_3$) δ −73.42 (s); ^{13}C NMR (100.7 MHz, CDCl$_3$) δ 171.7, 171.4, 120.3 (q, $J = 293.3$ Hz), 79.1–80.0 (m), 68.3, 60.2, 56.0, 54.9, 54.8, 54.0, 52.6, 52.2, 51.9, 46.7, 29.8, 14.3, 14.1; MS (MALDI-TOF) m/z 1203 ((M+H)$^+$, 100); HRMS (MALDI-TOF) calcd for C$_{37}$H$_{46}$F$_{27}$N$_4$O$_9$ 1203.2834, found 1203.2883.

F-DOTA 1

(2,2',2''-(10-(3-(1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yloxy)-2,2-bis ((1,1,1,3,3,3 hexa fluoro-2-(trifluoromethyl)propan-2-yloxy)methyl) prpyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid). To a solution of lithium hydroxide (1.4 g, 60.0 mmol) in water (10 mL) was added a solution of ester **6** (6.0 g, 5.0 mmol) in methanol (200 mL) at rt. The resulted mixture was stirred at rt. overnight. Then 2N HCl was added to the cloudy suspension to adjust the reaction mixture to pH 1. The solid was collected through centrifugation and then washed with water and diethyl ether. Removal of solvent under vacuum gave the product **1** as a white solid (5.4 g, 97% yield). ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.20 (s, 6H), 3.59 (s, 2H), 3.41 (s, 3H), 3.22 (br, 4H), 3.06 (br, 4H), 2.98 (s, 8H), 2.80 (br, 3H); ^{19}F NMR (376 MHz, CD$_3$OD) δ −70.93 (s); ^{13}C NMR (100.7 MHz, CD$_3$OD) δ 180.1,

177.4, 121.6 (q, J = 292.5 Hz), 81.0 (m), 69.9, 58.5, 56.8, 52.2, 51.9, 48.9, 45.9, 45.7, 22.2; MS (MALDI-TOF) m/z 1119 ((M+H)$^+$, 100); HRMS (CI) calcd for $C_{31}H_{36}F_{27}N_4O_9$ 1121.2051, found 1121.2792.

Alcohol 8

To a stirred solution of tetraethylene glycol **7** (97.0 g, 500.0 mmol) in tetrahydrofuran (450 mL) at 0 °C was added sodium hydride (60% in paraffin, 20.8 g, 520.0 mmol) slowly and the resulting mixture was stirred at rt. for 30 min. Then benzyl bromide (51.2 g, 300.0 mmol) was added and the resulting mixture was stirred at rt. overnight. After quenching the reaction with water (200 mL), the mixture was extracted with ethyl acetate (100 mL, 4 times). The combined organic phase was dried over anhydrous magnesium sulfate. After concentration under vacuum, the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate = 1/1) to give alcohol **8** as a clear oil (76.5 g, 90%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.28–7.29 (m, 5H), 4.51 (s, 2H), 3.52–3.66 (m, 16 H).

Mesylate 9

To a stirred solution of alcohol **8** (69.3 g, 243.7 mmol) and triethylamine (49.2 g, 68.4 mL, 487.0 mmol) in dichloromethane (700 mL) at 0 °C was added methanesulfonyl chloride (41.9 g, 28.3 mL, 365.6 mmol). The resulting mixture was stirred at rt. overnight and quenched with water (400 mL). The organic phase was collected and the aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with 2N HCl (100 mL), brine (100 mL) and dried over anhydrous magnesium sulfate. Concentrating the solution under vacuum gave the mesylate **9** as a clear oil (86.6 g, 98%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.25–7.32 (m, 5H), 4.54 (s, 2H), 4.32–4.34 (m, 2H), 3.71–3.73 (m, 2H), 3.59–3.66 (m, 12H), 3.03 (s, 3H).

Compound 10

A solution of alcohol **3** (5.9 g, 7.5 mmol) in tetrahydrofuran (50 mL) was stirred at 0 °C and potassium hydride (25% in paraffin, 1.4 g, 8.5 mmol) was added slowly to the solution. After the addition, the mixture was stirred for an additional 10 min at 0 °C and mesylate **9** (4.2 g, 7.5 mmol) was then added in one portion. The resulting mixture was stirred at rt. overnight and quenched with water (100 mL). The organic phase was collected and the aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with 2N HCl (100 mL) and brine (100 mL) and dried over anhydrous magnesium sulfate. Concentration under vacuum and flash chromatography on silica gel (*n*-hexane/ethyl acetate = 10/1) gave compound **10** as a clear oil (6.4 g, 80%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.26–7.33 (m, 5H), 4.56 (s, 2H), 4.07 (s, 6H), 3.54–3.69 (m, 16H), 3.45 (s, 2H); ^{19}F NMR (376 MHz, CDCl$_3$) δ −73.28 (s); ^{13}C NMR (100.7 MHz, CDCl$_3$) δ 138.3, 128.3, 127.7, 127.5, 120.1 (q, J = 292.6 Hz), 78.9–79.8 (m), 73.2, 70.7, 70.65, 70.6, 70.56, 70.3, 69.4, 66.3, 65.5, 46.2; MS (MALDI-TOF) m/z 1079 ((M+Na)$^+$, 100); HRMS (MALDI-TOF) calcd for $C_{32}H_{31}F_{27}NaO_8$ 1079.1458, found 1079.1517.

Alcohol 11

A suspension of compound **10** (6.0 g, 5.7 mmol) and palladium hydroxide (10%, 1.2 g) in methanol (80 mL) was stirred under an atmosphere of hydrogen for 2 h. After filtration, the mixture was concentrated under vacuum and purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate = 8/1) to give alcohol **11** as a clear oil (5.3 g, 97%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.99 (s, 6H), 3.53–3.60 (m, 16H), 3.39 (s, 2H).

Mesylate 12

To a stirred solution of alcohol **11** (5.1 g, 5.3 mmol) and triethylamine (3.2 g, 31.8 mmol) in dichloromethane (80 mL) at 0 °C was added methanesulfonyl chloride (1.9 g, 15.9 mmol). The

resulting mixture was stirred at rt. for 1h and quenched with water (50 mL). The organic phase was collected and the aqueous phase was extracted with ethyl acetate. The combined organic phase was dried over anhydrous magnesium sulfate. After concentration under vacuum, the residue was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate = 10/1) to give mesylate **12** as a clear oil (5.5 g, 99%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.34–437 (m, 2H), 4.04 (s, 6H), 3.73–3.76 (m, 2H), 3.53–3.67 (m, 12H), 3.43 (s, 2H), 3.04 (s, 3H).

Amine 13

A suspension of mesylate **12** (5.3 g, 5.1 mmol) and cyclen (1.8 g, 10.2 mmol) in a mixture of dimethylformamide (50 mL) was stirred at 60 °C overnight. After concentrating the reaction mixture to dryness under vaccum, the residue was purified by solid-phase extraction on FluoroFlash® silica gel (After loading the sample to fluorous silica gel, it was firstly washed with methanol/water (4/1 v/v, 200 mL) and then washed with methanol (400 mL). The product was yielded by concentrating the methanol eluant) to give the product **13** as a clear oil (5.2 g, 93% yield). ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.14 (s, 6H), 3.57–3.69 (m, 14H), 3.49 (s, 2H), 2.95–3.04 (m, 7H), 2.85–2.89 (m, 5H), 2.76–2.82 (m, 2H), 2.69 (s, 4H); ^{19}F NMR (376 MHz, CD$_3$OD) δ −71.14 (s); ^{13}C NMR (100.7 MHz, CD$_3$OD) δ 121.6 (q, J = 292.5 Hz), 80.5–81.4 (m), 78.3, 72.0, 71.9, 71.73, 71.7, 71.67, 71.6, 71.56, 71.5, 71.4, 71.41, 71.4, 71.2, 69.6, 67.5, 67.2, 50.9, 46.4, 44.4; MS (MALDI-TOF) *m/z* 1121 ((M+H)[+], 100); HRMS (MALDI-TOF) calcd for C$_{33}$H$_{44}$F$_{27}$N$_7$O$_4$ 1021.2779, found 1021.2790.

Tri-ethyl ester 14

To a stirred solution of amine **13** (5.1 g, 4.6 mmol) in tetrahydrofuran (25 mL) and dimethyl-formamide (25 mL) was added powdered anhydrous potassium carbonate (6.3 g, 46.0 mmol) and ethyl bromoacete (2.6 mL, 3.8 g, 23.0 mmol). The resulting mixture was stirred overnight at 60 °C. After filtration, the solvent was removed under vacuum and the residue was purified by flash chromatography on neutral aluminum oxide (CH$_2$Cl$_2$/Methanol = 10/1) to give the product **14** as a clear oil (5.2 g, 82% yield). ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.16–4.29 (m, 8H), 4.14 (s, 6H), 3.56–3.67 (m, 16H), 3.48 (s, 4H), 2.70–3.45 (m, 4H) 2.41–2.76 (m, 14H), 1.27–1.30 (m, 9H); ^{19}F NMR (376 MHz, CD$_3$OD) δ −71.12 (s); ^{13}C NMR (100.7 MHz, CD$_3$OD) δ 175.3, 175.1, 121.6 (q, J = 292.6 Hz), 80.7–81.5 (m), 71.9, 71.5, 71.4, 71.3, 71.2, 70.8, 68.5, 67.4, 67.1, 62.3, 62.26, 56.4, 56.1, 53.6, 51.9, 51.1, 48.4, 47.4, 14.5, 14.3; MS (MALDI-TOF) *m/z* 1379 ((M+H)[+], 100); HRMS (MALDI-TOF) calcd for C$_{45}$H$_{62}$F$_{27}$N$_4$O$_{13}$ 1379.3882, found 1379.3902.

F-DOTA 2

(2,2',2''-(10-(18,18,18-trifluoro-14,14-bis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl) propan-2-yloxy)methyl)-17,17-bis(trifluoromethyl)-3,6,9,12,16 pentaoxaoctadecyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid) Lithium hydroxide (0.9 g, 37.0 mmol) was added to a solution of compound **14** (5.1 g, 3.7 mmol) in methanol (100 mL) and water (10 mL). The resulting mixture was stirred at rt. for 8 h. Then 1N HCl was added to adjust the solution to pH 1. After removing the solvent under vacuum, the residue was purified by flash column chromatography on neutral aluminum oxide (CH$_2$Cl$_2$/Methanol = 10/1) to give compound **2** as a white solid (4.7 g, 99%). ^{1}H NMR (400 MHz, Acetone-*d6*) δ 4.20 (s, 6H), 3.53–3.59 (m, 16H), 2.2–3.2 (m, 24H); ^{19}F NMR (376 MHz, CD$_3$OD) δ −71.15 (s); ^{13}C NMR (100.7 MHz, CD$_3$OD) δ 180.9, 179.6, 175.3, 175.1, 121.5 (q, J = 292.6 Hz), 80.5–81.4 (m), 71.9, 71.6, 71.5, 71.4, 71.3, 69.9, 68.8, 67.5, 67.1, 60.6, 59.2, 53.9, 52.4, 51.9, 47.4; MS (MALDI-TOF) *m/z* 1295 ((M+H)[+], 100); HRMS (MALDI-TOF) calcd for C$_{39}$H$_{50}$F$_{27}$N$_4$O$_{13}$ 1295.2943, found 1295.2953.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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9. Since *F*-DOTA **1** and *F*-DOTA **2** each contains three carboxylic groups, the protonation status of which is pH-dependent, the 1-octanol/water partition measurements were conducted with physiological saline buffer (PBS, 50 mM phosphate, 100 mM NaCl, 1 mM EDTA, pH 7.0) as the aqueous phase. Specifically, *F*-DOTA **1** or *F*-DOTA **2** (5 mg) was dissolved in mixture of PBS buffer (0.5 mL) and 1-octanol (0.5 mL). The sample was put into an Eppendorff® microcentrifuge tube (1.5 mL), which was then taped to a type 16700 mixer from Thermolyne to be shaken vigorously for 20 min. After phase separation, the aqueous phase and 1-Octanol phases were taken out for ^{19}F NMR, respectively, with a sealed capillary of 1% hexanfluorobenzene in methanol-*d4* as the internal standard. P_{oct} was calculated based on the ratio of ^{19}F NMR signal areas in the 1-octanol and water.

10. T_1 and T_2 of of the trifluoromethyl group of perfluorooctyl bromide were measured under the same condition as for *F*-DOTA **1** and *F*-DOTA **2** by ^{19}F NMR (376 MHz) in methanol-*d4* at a concentration of 0.025 M.
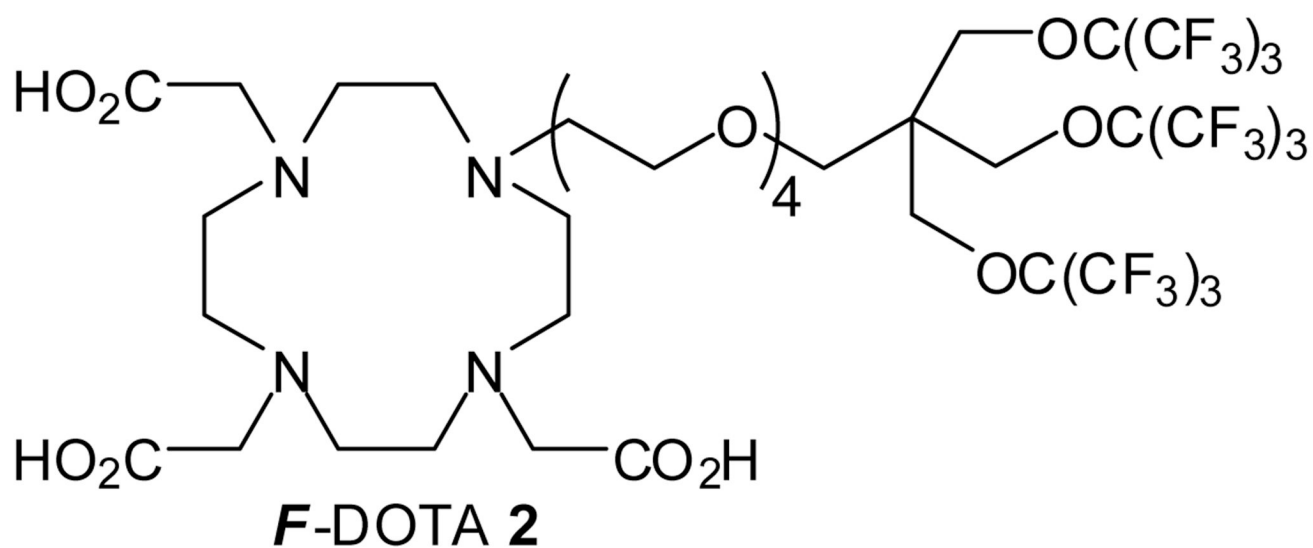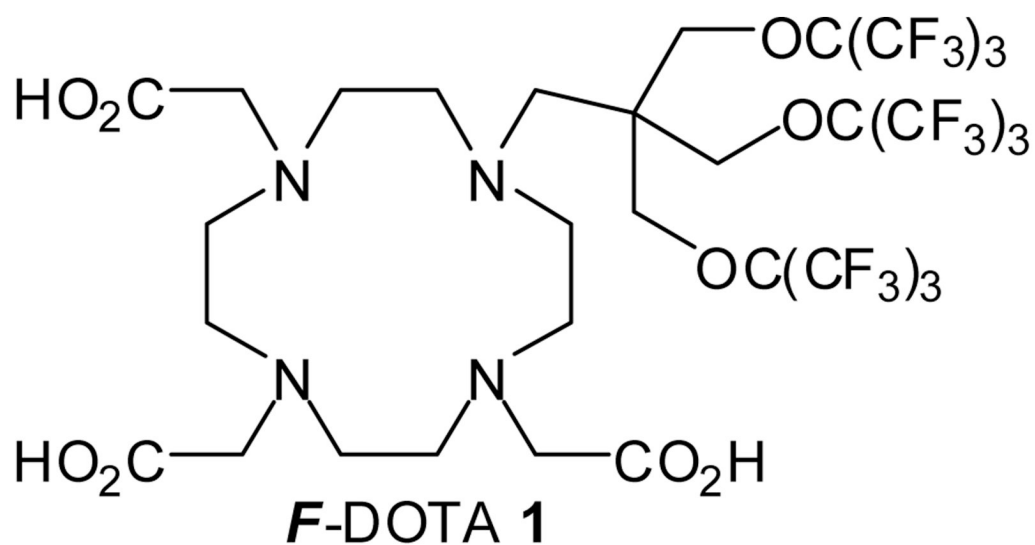
Figure 1.
Structures of target molecules *F*-DOTA **1** and *F*-DOTA **2**. The fluorocarbon moiety of both **1** and **2** has spherical symmetry.
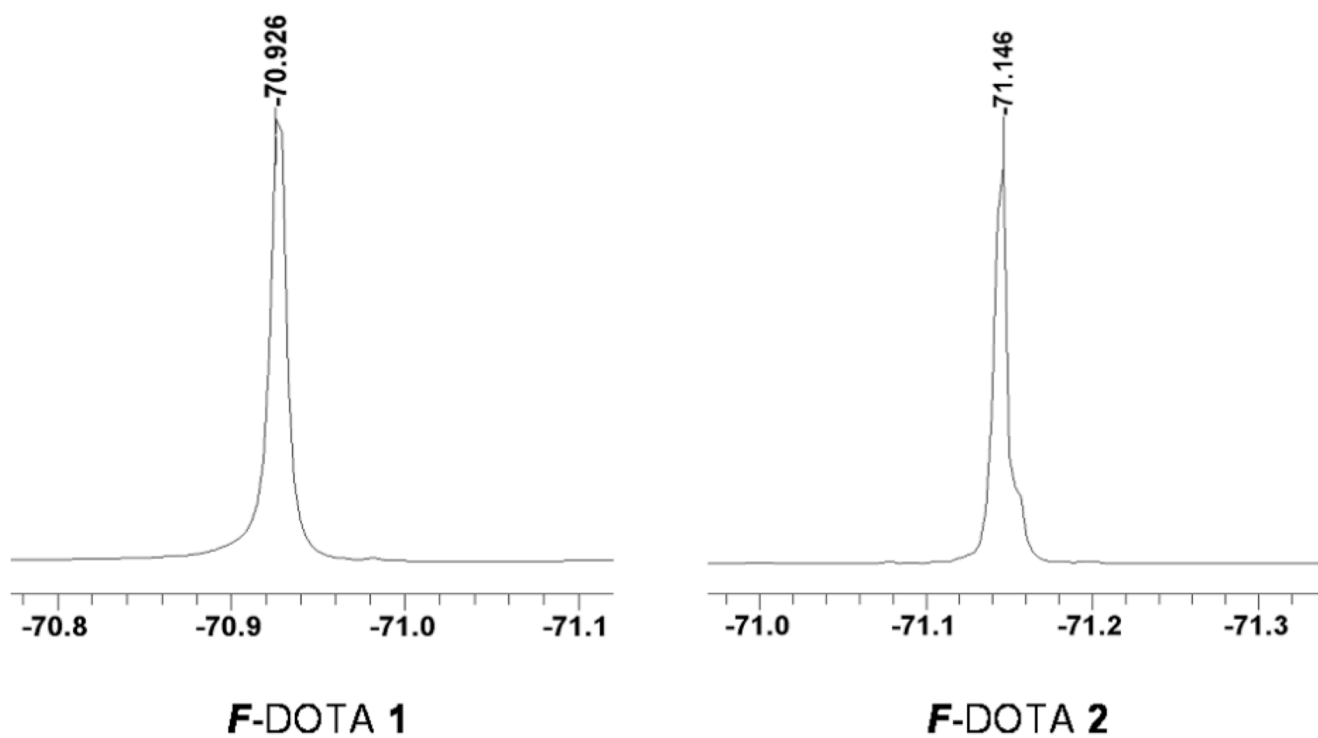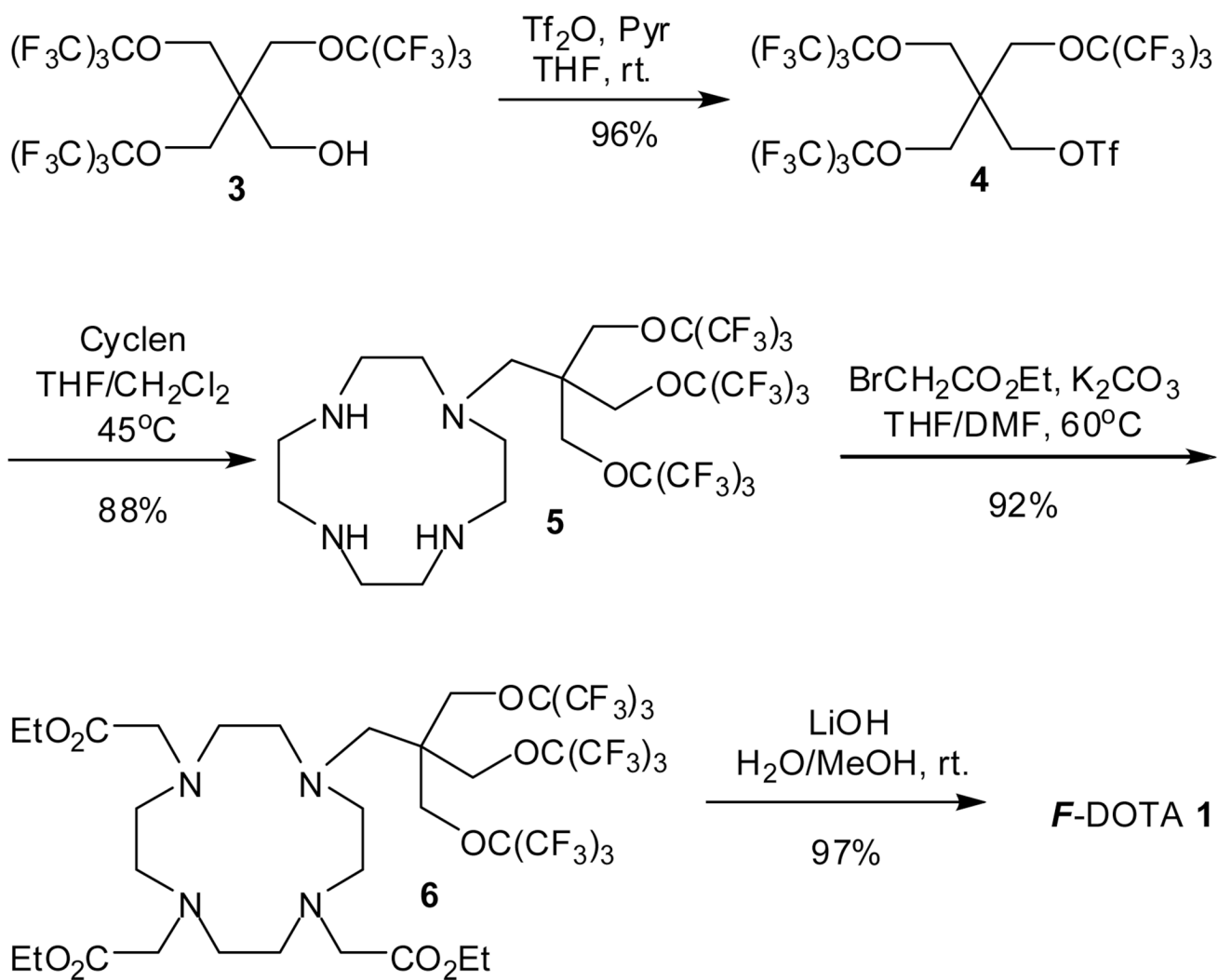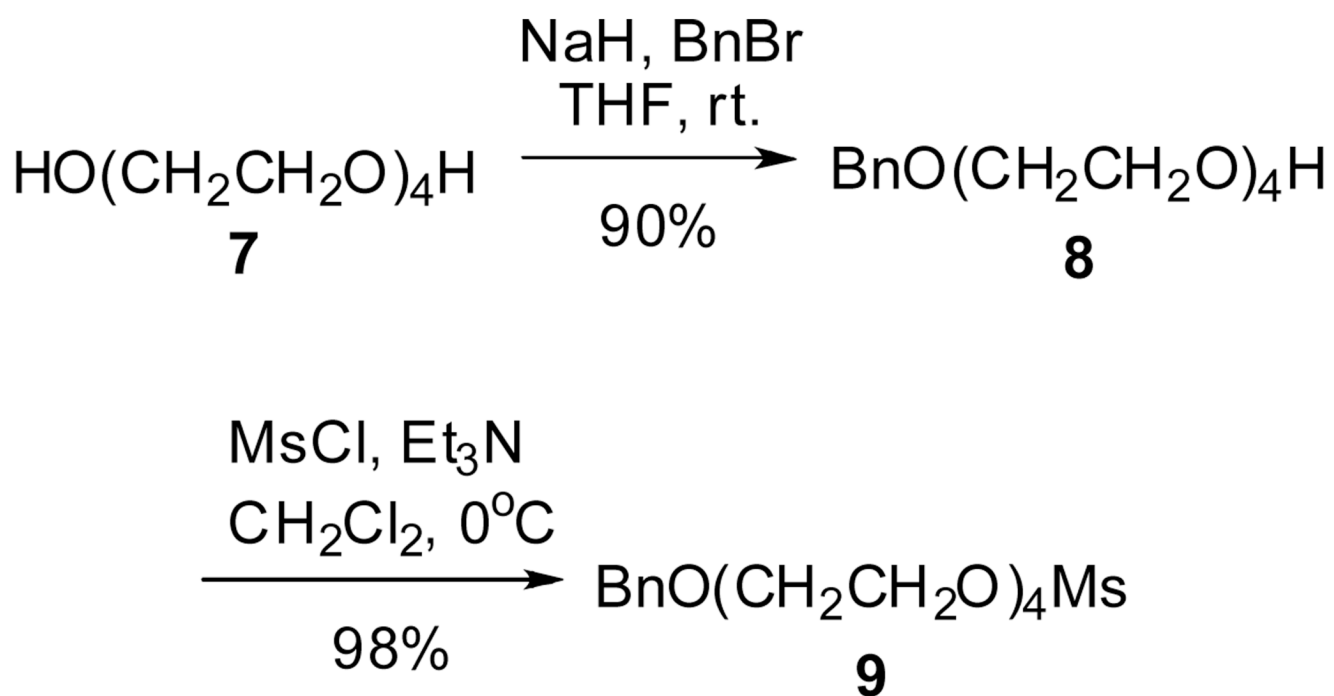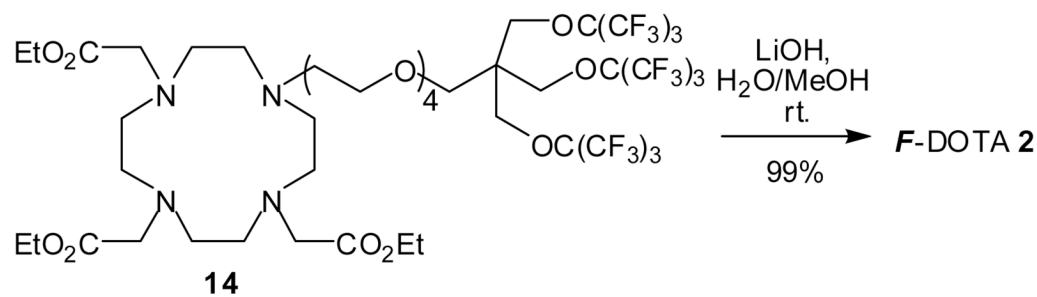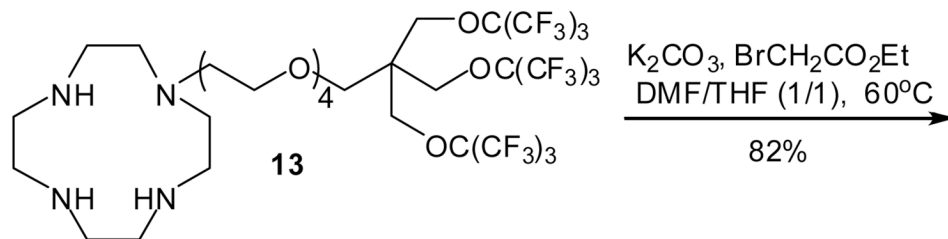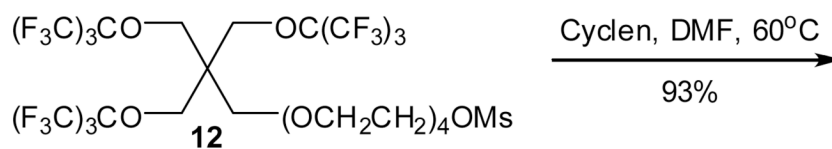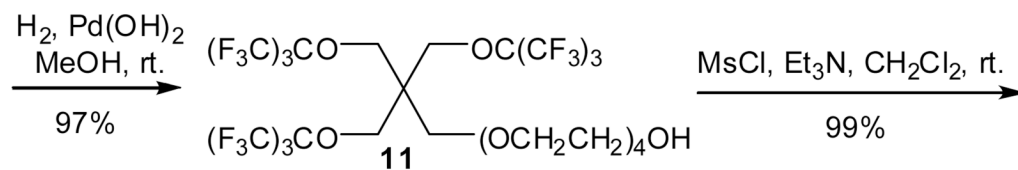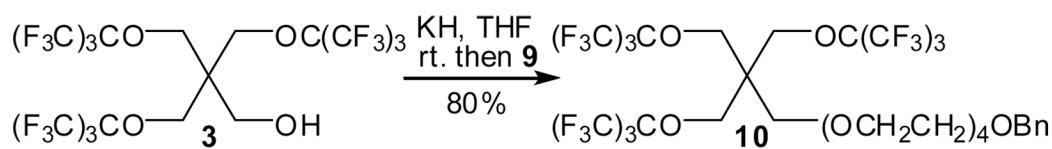
Figure 2.
^{19}F NMR spectra of *F*-DOTA **1** and *F*-DOTA **2** (0.025 M in CD$_3$OD, 376 MHz).

Scheme 1.
Synthesis of *F*-DOTA **1**.

$$\text{HO(CH}_2\text{CH}_2\text{O)}_4\text{H} \quad \xrightarrow[90\%]{\begin{array}{c}\text{NaH, BnBr}\\ \text{THF, rt.}\end{array}} \quad \text{BnO(CH}_2\text{CH}_2\text{O)}_4\text{H}$$

7   **8**

$$\xrightarrow[98\%]{\begin{array}{c}\text{MsCl, Et}_3\text{N}\\ \text{CH}_2\text{Cl}_2,\ 0\,^{\circ}\text{C}\end{array}} \quad \text{BnO(CH}_2\text{CH}_2\text{O)}_4\text{Ms}$$

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Scheme 2.
Preparation of mesylate **9**.

Scheme 3.
Synthesis of *F*-DOTA **2**.