The Design and Synthesis of Highly Branched and Spherically Symmetric Fluorinated Macroyclic 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-oxylene 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.

Key words: 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 $^{195}$Gd$^{3+}$ for contrast-enhanced $^1$H MR imaging (See Figure 1 of reference 1). The macrocyclic 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 chose the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacacetate) 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 macrocyclic 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-oxylene, as oxylene is hydrophilic and biocompatible. One such chelator with a tetra-oxylene linker ($F$-DOTA 2) is presented in Figure 1. The fluorocarbon moieties of both molecules have spherical symmetry.

Figure 1  Structures of target molecules $F$-DOTA 1 and $F$-DOTA 2. The fluorocarbon moiety of both 1 and 2 has 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 trifluromethanesulfonyl anhydride in the presence of pyridine gave triflate 4 in excellent yield, which was isolated through phase separation after the addition of 10% water to the reaction mixture. It is noteworthy that tetrahydrofuran, instead of the commonly used dichloromethanes 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 an excess of trifluromethanesulfonic 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) at 45 °C overnight. The product 5 was extracted with FC-72 (perfluorohexanes) from the dried reaction residue (dissolved in dichloromethane) in 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) at 60 °C provided ester 6 in 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 molecule F-DOTA 1 in 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 in good yield on a 50-gram scale from the commercially available tetraethylene glycol 7 by selectively protecting one of the hydroxyl groups with benzyl bromide3 and transforming the other hydroxyl group into mesylate 9. It is an improvement over our former synthesis 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 as follows (Scheme 3). The highly fluorinated alcohol 3 was first attached to the hydrophilic tetra-oxyethylene linker 9 to afford compound 10 in good yield. Then, removal of the benzyl group in compound 10 by palladium hydroxide catalyzed hydrogenolysis gave alcohol 11 in an excellent yield, which was then treated with methanesulfonyl chloride and triethylamine to give the mesylate 12 in 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 provided amine 13 in 93% yield. Compound 13 was then reacted with ethyl bromoacetate to afford ester 14 in good yield. Finally, treatment of compound 14 with lithium hydroxide gave the target molecule F-DOTA 2 in 99% yield.

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

The flexible oligo-oxyethylene linker is the variable portion of our chelator design. Hence, its impact on the phys-

Scheme 1   Synthesis of F-DOTA 1

Scheme 2   Preparation of mesylate 9

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 spectroscopy.$^9$ $P_{oct}$ of $F$-DOTA$_1$ and $F$-DOTA$_2$ are 7.63 and 1.40 $\times 10^{-2}$, 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-$d_4$ 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-$d_4$ 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.

$^1$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 THF (250 mL) was added dropwise a solution of trifluoroacetanilide (8.1 mL, 14.1 g, 50.0 mmol) in THF (20 mL) at 0 °C. After stirring at this temperature for 1 h, the reaction was quenched by the slow addition of H2O (27 mL). The mixture was then transferred to a separatory funnel and the lower phase was collected. Washing the oil with CH2Cl2 (10 mL) gave the pure triflate 4 as a clear oil; yield: 8.8 g (96%). This compound is unstable at r.t. and needs to be used immediately after preparation.

\[ \text{Triflate 4} \]

\[ \text{Amine 5} \]

A suspension of triflate 4 (8.5 g, 9.3 mmol) and cyclen (3.3 g, 18.9 mmol) in a mixture of THF and CH2Cl2 (50 mL/50 mL) was stirred at r.t. for 2 h. The temperature was slowly raised to 45 °C and the mixture was then stirred overnight at this temperature. After concentration the reaction mixture to dryness under vacuum, the residue was dissolved in CH2Cl2 (50 mL) and extracted with FC-72 (3 × 50 mL). Evaporation of the combined FC-72 phases gave the product 5 as a clear oil; yield: 7.7 g (88%).

\[ \text{Amine 5} \]

\[ \text{Triethyl Ester 6} \]

Powdered K2CO3 (8.3 g, 60.2 mmol) and ethyl bromoacetate (4.2 g, 68.4 mL, 487.0 mmol) in CH2Cl2 (700 mL) at 0 °C was added MsCl (41.9 g, 28.3 mL, 365.6 mmol). The resulting mixture was stirred for 10 min at 0 °C. To a solution of LiOH (1.4 g, 60.0 mmol) in H2O (10 mL) was added methyl 1,4,7-triyl[triaicetic acid (CH2Cl2–MeOH, 10:1) to give the product 6 as a clear oil; yield: 6.3 g (92%).

\[ \text{Triethyl Ester 6} \]

1H NMR (400 MHz, CDCl3); \( \delta = 4.03–4.10 \) (m, 12 H), 3.34 (s, 2 H), 3.25 (s, 4 H), 2.62–2.76 (m, 18 H), 1.16–1.21 (m, 9 H).

13C NMR (100.7 MHz, CDCl3); \( \delta = 171.7, 171.4, 120.3 \) (q, \( J = 293.3 \) Hz), 79.1–80.0 (m, 683), 54.3, 54.3, 46.9, 46.0, 45.7, 45.5.

19F NMR (376 MHz, CDCl3); \( \delta = –73.31 \) (s).

HRMS (MALDI-TOF); \( m/z = 945 \) (45 M + H\(^+\)), 100). Concentration of the solution under vacuum gave the mesylate 9 as a clear oil; yield: 86.6 g (98%).

\[ \text{Mesylate 9} \]

A solution of alcohol 3 (5.9 g, 7.5 mmol) in THF (50 mL) was stirred at 0 °C and KH (25% in paraffin, 4.8 g, 85 mmol) was added slowly to the solution. After the addition, the mixture was stirred for an additional 10 min at 0 °C and the mesylate 9 (4.2 g, 7.5 mmol) was then added in one portion. The resulting mixture was stirred overnight at r.t. and quenched with H2O (100 mL). The organic phase was collected and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with aq 2 N HCl (100 mL), brine (100 mL), and dried (MgSO4). Concentration under vacuum and flash chromatography on silica gel (n-hexane–EtOAc, 1:1) gave the compound 10 as a clear oil; yield: 6.4 g (80%).

\[ \text{Compounds} \]

To a stirred solution of alcohol 3 (5.9 g, 7.5 mmol) in THF (50 mL) was stirred at 0 °C and KH (25% in paraffin, 4.8 g, 85 mmol) was added slowly to the solution. After the addition, the mixture was stirred for an additional 10 min at 0 °C and the mesylate 9 (4.2 g, 7.5 mmol) was then added in one portion. The resulting mixture was stirred overnight at r.t. and quenched with H2O (100 mL). The organic phase was collected and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with aq 2 N HCl (100 mL), brine (100 mL), and dried (MgSO4). Concentration under vacuum and flash chromatography on silica gel (n-hexane–EtOAc, 10:1) gave the compound 10 as a clear oil; yield: 6.4 g (80%).

For the synthesis, see the original report. Synthesis 2008, No. 2, 215–220 © Thieme Stuttgart · New York
Alcohol 11
A suspension of compound 10 (6.0 g, 5.7 mmol) and Pd(OH)$_2$ (10%, 1.2 g) in MeOH (80 mL) was stirred under H$_2$ for 2 h. After filtration, the mixture was concentrated under vacuum and purified by flash chromatography on silica gel (n-hexane–EtOAc, 8:1) to give the alcohol 11 as a clear oil; yield: 5.3 g (97%).

1H NMR (400 MHz, CDCl$_3$): $\delta = 3.99$ (s, 6 H), 3.53–3.60 (m, 16 H), 3.39 (s, 2 H).

Mesylate 12
To a stirred solution of alcohol 11 (5.1 g, 5.3 mmol) and Et$_3$N (3.2 g, 31.8 mmol) in CH$_2$Cl$_2$ (50 mL) at 0 °C was added MsCl (1.9 g, 31.8 mmol) in CH$_2$Cl$_2$ (80 mL). The resulting mixture was stirred for 1 h at r.t. and quenched with H$_2$O (50 mL). The organic phase was collected and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were dried (MgSO$_4$). After concentration under vacuum, the residue was purified by flash chromatography on silica gel (n-hexane–EtOAc, 10:1) to give the mesylate 12 as a clear oil; yield: 5.5 g (99%).

1H NMR (400 MHz, CD$_3$OD): $\delta = 4.34–437$ (m, 2 H), 3.39 (s, 2 H).

Amine 13
A suspension of mesylate 12 (5.3 g, 5.1 mmol) and cyclen (1.8 g, 6.0 g, 5.7 mmol) and Pd(OH)$_2$ (5.3 g, 5.1 mmol) and ethyl bromoacetate (2.6 mL, 3.8 g, 23.0 mmol). The reaction mixture was stirred for 8 h at r.t. Then aq 1 N 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$_2$–MeOH, 10:1) to give the compound 2 as a white solid; yield: 4.7 g (99%).

1H NMR (400 MHz, acetone-$d_6$): $\delta = 4.20$ (s, 6 H), 3.53–3.59 (m, 16 H), 2.2–3.2 (m, 24 H).

13C NMR (100.7 MHz, CD$_3$OD): $\delta = 121.6$ (q, $J = 292.5$ Hz), 80.5–81.4 (m), 78.3, 72.0, 71.9, 71.7, 71.7, 71.6, 71.5, 71.4, 71.4, 71.2, 69.6, 67.5, 67.2, 50.9, 46.4, 44.4.

19F NMR (376 MHz, CD$_3$OD): $\delta = –71.14$ (s).

MS (MALDI-TOF): $m/z = 1295$ ([M + H]$^+$), 100.

HRMS (MALDI-TOF): $m/z = 1295.2943$; calcd for C$_{39}$H$_{50}$F$_{27}$N$_4$O$_{13}$: 1295.2943; found: 1295.2943; 1295.2953.

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References
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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/H$_2$O 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 Eppendorf®
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% hexafluorobenzene in MeOH-
$\text{d}_4$ as the internal standard. $P_{\text{oct}}$ was calculated based on the
ratio of $^{19}$F NMR signal areas in the 1-octanol and H$_2$O.
(10) $T_1$ and $T_2$ 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 spectroscopy (376
MHz) in MeOH-$\text{d}_4$ at a concentration of 0.025 M.