Published in final edited form as:

Chem Commun (Camb). 2011 July 7; 47(25): 7233–7235. doi:10.1039/c1cc11150g.

Fluorinated Paramagnetic Chelates as Potential Multi-chromic ^{19}F Tracer Agents

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Abstract

A class of potential multi-chromic ^{19}F imaging tracers is made by pairing metal ions with a fluorinated chelator. All fluorinated metal chelates emit a single ^{19}F signal. Paramagnetic metal ions shifted the ^{19}F signal frequency and made the ^{19}F relaxation rates insensitive toward local chemical environment.

^1H and ^{19}F are respectively the most and 2nd most sensitive stable nuclei for magnetic resonance spectroscopy and imaging (MRS/I), each with a nuclear spin of $\frac{1}{2}$. Although ^1H MRI has wide clinical usage, ^{19}F MRI, is hardly used in medicine due to lack of suitable ^{19}F imaging agents. Recently, there is a renewed interest in ^{19}F MRI due to the need to track therapeutic agents (genes, cells, etc.) and to monitor biochemical reactions *in vivo* non-invasively.^{1–10} Compared with ^1H , the ^{19}F signal has negligible endogenous background and a much wider frequency range (~ 400 ppm) and thus is ideally suited for tracking objects and monitoring reactions *in vivo*. For various biomedical applications, it is highly desirable to monitor several objects simultaneously with ^{19}F MRS/I. This requires a set of ^{19}F imaging tracers, each emitting the ^{19}F signal as a specific radio frequency.

One way to achieve ^{19}F multi-chromicity is to use different fluorinated compounds that have distinct ^{19}F chemical shifts.¹¹ This is essentially a “one synthesis, one compound, one frequency” approach, which is synthetically inefficient. Here, we develop a “one synthesis, one set of compounds, multiple frequencies” approach, which is synthetically much more efficient. This is achieved by synthesizing a fluorinated chelator **FC** (Fig. 1) and then pairing **FC** with various paramagnetic metal ions (M^{x+}). Paramagnetic metal ions have unpaired electrons whose magnetic moments can greatly influence nearby nuclear spins.^{12–14} **FC** contains nine chemically identical fluorine atoms as the ^{19}F signal emitter while the paramagnetic ion acts as the ^{19}F signal modulator. This approach has several advantages. First, by synthesizing just one fluorinated chelator, multiple ^{19}F signal frequencies can be generated. Second, paramagnetic ions can shorten the relaxation times of nearby nuclei. Shorter ^{19}F relaxation times can lead to higher signal/noise ratio.^{15–17} Third, paramagnetic ions can dominate the ^{19}F relaxation times of fluorinated metal chelates and thus reduce their dependency on the local environment. Fourth, as a result of their chemical

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[†]Electronic Supplementary Information (ESI) available: [General information, synthesis of **FC**, NMR and MS spectra and HPLC chromatograms of intermediates and chelates]. See DOI: 10.1039/b000000x/

similarity, different FC-M^{x+} will cause similar perturbation to the biodistribution of the various objects that they label.

With these ideas in mind, a convenient synthesis of **FC** on a 5-g scale was developed (Supplementary Information). A set of di- and tri-valent metal ions, was easily chelated by **FC**. All these metal ions can bind to the macrocyclic chelator in **FC** with high affinity, with Bi^{3+} being the strongest and Ca^{2+} the weakest.¹⁸ The chelates were purified by HPLC using fluorinated columns. **FC** and all the metal chelates have excellent aqueous solubility due to low fluorine content (21.4% for **FC**). In contrast, two fluorinated chelators we previously synthesized had low aqueous solubility due to higher fluorine contents (39.6 & 45.9%).¹⁹

As expected, a frequency spread of 3708 Hz (7.89 ppm) was produced by pairing **FC** with different metal ions at the concentration of 50 mM (Fig. 2a). In all cases, there is a single unsplit ^{19}F signal. In a previous report on fluorinated lanthanide chelates, multiple ^{19}F signals were observed.¹⁰ This difference is likely the result of the flexible linker between the fluorocarbons and the metal chelate. Diamagnetic ions (black bars), Bi^{3+} , Hg^{2+} , Y^{3+} , Pb^{2+} , La^{3+} , Ca^{2+} and Cd^{2+} , caused very little change in the ^{19}F chemical shift relative to **FC** (empty bar). Among the paramagnetic ions (gray bars), Fe^{3+} , Gd^{3+} , Er^{3+} , Dy^{3+} , Ho^{3+} and Tb^{3+} caused significant ^{19}F chemical shift change with Tb^{3+} being the most effective. Fig. 3 plots the ^{19}F spectra of **FC-Tb**³⁺, **FC-Gd**³⁺, **FC-Y**³⁺ and **FC**. Some metal ions, especially paramagnetic ions, also shorten the ^{19}F relaxation times of the fluorinated metal chelates (Fig. 2b). Among the diamagnetic ions, only Bi^{3+} shortens ^{19}F T_1 and T_2 significantly. The paramagnetic ions shorten the ^{19}F relaxation times more effectively and can be classified into three groups. For Sm^{3+} , Ce^{3+} , Eu^{3+} , Pr^{3+} , Nd^{3+} and Yb^{3+} , the relaxation time reduction is modest. In the case of Sm^{3+} , ^{19}F T_1 and T_2 actually increased slightly, as is the case with the diamagnetic Hg^{2+} and Y^{3+} . For Dy^{3+} , Tb^{3+} , Ho^{3+} and Er^{3+} , the relaxation time reduction is dramatic, around 95%. For Gd^{3+} , Fe^{3+} , Ni^{3+} and Cu^{2+} , the relaxation time reduction is overwhelming, around 99%. As expected, the most effective metal ion to shorten the ^{19}F relaxation times is Gd^{3+} , which reduces T_1 and T_2 to 4.2 ms and 1.7 ms in **FC-Gd**³⁺, respectively, from 1330 ms and 701 ms in **FC**.

Then a study was carried out to see whether the intramolecular paramagnetic ion makes ^{19}F T_1 and T_2 insensitive toward oxygen. Oxygen is paramagnetic and is known to reduce ^{19}F relaxation times both *in vitro*²⁰ and *in vivo*.²¹ To test the effect of oxygen, ^{19}F T_1 and T_2 were measured in solutions saturated with N_2 and O_2 , respectively. It is found that, as expected, the paramagnetic oxygen resulted in dramatic reduction in ^{19}F relaxation times in most cases (Fig. 2c). However, in the case of Tb^{3+} , Ho^{3+} , Dy^{3+} , Er^{3+} , Gd^{3+} , Fe^{3+} , Ni^{3+} and Cu^{2+} , the relaxation time reduction caused by saturated oxygen is 5% or less. It is interesting to note that the diamagnetic Bi^{3+} also stabilizes T_1 and T_2 against oxygen.

Encouraged by this result, studies were carried out to verify whether the ^{19}F signal frequency and relaxation times of paramagnetic fluorinated chelates have weaker dependency than those of diamagnetic fluorinated chelates on pH, temperature, serum protein and human sera. Here, the paramagnetic **FC-Tb**³⁺ and **FC-Gd**³⁺ are compared with the diamagnetic **FC-Y**³⁺ and **FC**. The results are summarized in Tables 1 and 2. First, pH, temperature, bovine serum albumin (BSA) and human sera produced little change in ^{19}F chemical shift in both para- and diamagnetic compounds ($\Delta\delta < 1$ ppm in all cases except for **FC-Tb**³⁺ in 10% BSA, where $\Delta\delta = 1.9$ ppm). Second, pH, temperature, BSA and human sera produced much smaller change ^{19}F relaxation times in the two paramagnetic compounds than in the two diamagnetic compounds. Thus, the intra-molecular paramagnetic ion indeed drastically shortens the ^{19}F relaxation times and makes them rather insensitive toward environmental fluctuations.

Conclusions

A “one synthesis, one set of compounds, multiple frequencies” approach has been developed for making fluorinated metal chelates. In such a fluorinated metal chelate, the fluorocarbon sphere is the ^{19}F signal emitter while the metal chelate is the ^{19}F signal modulator, which plays three roles: shifting ^{19}F resonance frequency, which enables collecting ^{19}F signals at different radio frequencies; reducing ^{19}F relaxation times, which can improve the signal/noise ratio; dominating the ^{19}F relaxation process, which makes the ^{19}F signal insensitive toward environmental fluctuations. These features make fluorinated paramagnetic chelates potential tracer agents for multi-chromic ^{19}F MRS/I.

The work presented here is just a proof of concept. Further studies, such as introducing more fluorine atoms to improve signal intensity, varying the ^{19}F - M^{x+} distance and the $^{19}\text{F}/\text{M}^{x+}$ ratio to generate larger ^{19}F frequency shift and to further reduce the environment-dependency of relaxation times, are under active development.

A potential clinical application of multi-chromic ^{19}F tracers is to use them to label different therapeutic agents, such as cells, antibodies and drug delivery vehicles. By labelling different objects with different fluorinated metal chelates, each object can be tracked at a specific radio frequency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Maryland Department of Business & Economic Development & NIH grant EB004416.

Notes and references

1. Janjic JM, Srinivas M, Kadayakkara DKK, Ahrens ET. J. Am. Chem. Soc. 2008; 130:2832–2841. [PubMed: 18266363]
2. Mizukami S, Takikawa R, Sugihara F, Kori Y, Tochio H, Wälchi M, Shirakawa M, Kikuchi K. J. Am. Chem. Soc. 2008; 130:794–795. [PubMed: 18154336]
3. Thurecht KJ, Blakey I, Peng H, Squires O, Hsu S, Alexander C, Whittaker AK. J. Am. Chem. Soc. 2010; 132:5336–5337. [PubMed: 20345132]
4. Gianolio E, Napolitano R, Fedeli F, Arena F, Aime S. Chem. Commun. 2009:6044–6046.
5. Kenwright AM, Kuprov I, De Luca E, Parker D, Pandya SU, Senanayake PK, Smith DG. Chem. Commun. 2008:2514–2516.
6. Reid DG, Murphy PS. Drug Disc. Today. 2008; 13:473–480.
7. Ruiz-Cabello J, Walczak P, Kedziorek DA, Chacko VP, Schmieder AH, Wickline SA, Lanza GM, Bulte JWM. Magn. Reson. Med. 2008; 60:1506–1511. [PubMed: 19025893]
8. Higuchi M, Iwata N, Matsuba Y, Sato K, Sasamoto K, Saido TC. Nat. Neurosci. 2005; 8:527–533. [PubMed: 15768036]
9. Jiang Z-X, Liu X, Jeong E-K, Yu YB. Angew. Chem. Int. Ed. 2009; 48:4755–4758.
10. Chalmers KH, Luca ED, Hogg NHM, Kenwright AM, Kuprov I, Parker D, Botta M, Wilson JI, Blamire AM. Chem. Eur. J. 2010; 16:134–148. [PubMed: 19957317]
11. Partlow KC, Chen J, Brant JA, Neubauer AM, Meyerrose TE, Creer MH, Nolta JA, Caruthers SD, Lanza GM, Wickline SA. FASEB J. 2007; 21:1647–1654. [PubMed: 17284484]
12. Barry CD, North AC, Glasel JA, Williams RJP, Xavier AV. Nature. 1971; 232:236–245. [PubMed: 4937076]
13. Sanders JK, Williams DH. Nature. 1972; 240:385–390.

14. Peters JA, Huskens J, Raber DJ. *Prog. NMR Spectrosc.* 1996; 28:283–350.
15. Ratner AV, Quay S, Muller HH, Simpson BS, Hurd R, Young SW. *Invest. Radiol.* 1989; 24:224–227. [PubMed: 2753638]
16. Lee H, Price RR, Holburn GE, Partain CL, Adams MD, Cachiers WP. *J. Magn. Reson. Imag.* 1994; 4:609–613.
17. Neubauer AM, Myerson J, Caruthers SD, Hockett FD, Winter PM, Chen J, Gaffney PJ, Robertson JD, Lanza GM, Wickline SA. *Magn. Reson. Med.* 2008; 60:1066–1072. [PubMed: 18956457]
18. Viola-Villegas N, Doyle RP. *Coord. Chem. Rev.* 2009; 253:1906–1925.
19. Jiang Z-X, Yu YB. *Synthesis.* 2008:215–220. [PubMed: 20585414]
20. Parhami P, Fung BM. *J. Phys. Chem.* 1983; 87:1928–1931.
21. Shukla HP, Mason RP, Woessner DE, Antich PP. *J. Magn. Reson. B.* 1995; 106:131–141.

Fig.1.
Structure of a fluorinated chelator **FC**

Fig.2.

Effects of metal ions on the ^{19}F signal frequency (**a**, upper) in terms of chemical shift in ppm unit; relaxation times (**b**, middle; left: T_1 ; right: T_2); and relaxation time reduction caused by O_2 (**c**, lower; left: T_1 ; right: T_2). Each sample contains 50 mM fluorinated metal chelate in phosphate-buffered saline (PBS), pH 7.0, 25°C. All samples are bubbled with a flow of pure N_2 (for **a**, **b** and **c**) or O_2 (for **c**) for 10 min at 0°C before measurement. The relaxation time reduction is defined as: $[T_i(\text{N}_2) - T_i(\text{O}_2)] / T_i(\text{N}_2) \times 100\%$, with $i = 1$ or 2. For experimental details, see Supplementary Information.

Fig.3.
 ^{19}F NMR spectra of 50 mM of **FC-Tb $^{3+}$** , **FC-Gd $^{3+}$** , **FC-Y $^{3+}$** , **FC** at pH7.0, 25°C in PBS saturated with N_2 .

Table 1

pH and temperature effects on the ^{19}F chemical shift and relaxation times.²² All samples contain 50 mM fluorinated metal chelate in PBS after bubbled with a flow of pure N_2 for 10 min at 0°C before measurement. The changes are relative to the same sample at pH7, 25°C in PBS. For accuracies of T_1 and T_2 measurements, see Table S1 of Supplemental Information.

Changed parameters	Chelates	pH6	pH8	10°C	35°C	45°C
$\Delta\delta$ (ppm)	FC-Tb $^{3+}$	0.2	0.2	0.6	-0.4	-0.8
	FC-Gd $^{3+}$	0.1	0.6	0.5	-0.1	-0.1
	FC-Y $^{3+}$	0	0	0	-0.1	-0.1
	FC	0	0	0.1	-0.1	-0.1
ΔT_1 (ms)	FC-Tb $^{3+}$	0.7	-0.9	-6.6	3.3	8.7
	FC-Gd $^{3+}$	-0.3	-0.2	-0.1	-0.2	-0.3
	FC-Y $^{3+}$	-83	-16	-462	239	619
	FC	101	110	-348	-15	50
ΔT_2 (ms)	FC-Tb $^{3+}$	4.8	5.1	-4.7	1.2	4.3
	FC-Gd $^{3+}$	-0.6	-0.6	-0.3	-0.4	-0.4
	FC-Y $^{3+}$	451	413	-63	748	728
	FC	89	125	-30	7	-82

Table 2

The effect of serum proteins on the ^{19}F chemical shift and relaxation times.²² All samples contain 50 mM fluorinated metal chelate in either 10% of BSA in PBS or human sera at 25°C. Samples were bubbled with a flow of pure N_2 or O_2 for 10 min at 0°C before measurement. The changes are relative to the same sample at pH7, 25°C in PBS bubbled with pure N_2 for 10 min.

Changed parameters	Chelates	10% BSA in PBS	Human sera	
			Under N_2	Under O_2
$\Delta\delta$ (ppm)	FC-Tb³⁺	1.9	0.1	0.1
	FC-Gd³⁺	0.8	0.3	0.3
	FC-Y³⁺	0	-0.1	0
	FC	0	0	0.1
ΔT_1 (ms)	FC-Tb³⁺	-2.5	7.1	2.5
	FC-Gd³⁺	0.3	0	0.2
	FC-Y³⁺	-314	-303	-718
	FC	-219	-83	-490
ΔT_2 (ms)	FC-Tb³⁺	-31.1	-29.8	-30.9
	FC-Gd³⁺	-1.1	-0.8	-1.0
	FC-Y³⁺	-644	-569	-593
	FC	-600	-492	-487