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Synthesis of two persistent fluorinated tetrathiatriarylmethyl (TAM) radicals for biomedical EPR applications

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ABSTRACT

Tetrathiatriarylmethyl radicals are attractive spin probes extensively used in biomedical magnetic resonance applications. We report a straightforward synthesis of two original tetrathiatriarylmethyl radicals incorporating, respectively, 15 and 45 fluorine atoms, and thus possessing a high affinity to fluorous media. **F15T-03** and **F45T-03** exhibit a single sharp EPR spectrum and their EPR line broadening is highly sensitive to molecular oxygen. These spin probes are specially designed for assessment of tumor oxygenation using perfluorocarbon formulations.

Molecular oxygen plays one of the most important roles in the metabolism of living organisms. Abnormal tissue oxygenation is closely linked to number of diseases (e.g., cancer, stroke, ischemic diseases).[1] Therefore, it is of particular importance for pO_2 in vitro and in vivo assessments to rely upon accurate methods. The techniques for measuring oxygen partial pressure in biological media include both non magnetic and magnetic resonance (MR) based methods. Typically polarographic oxygen electrodes, fluorescence quenching, phosphorescence quenching, near infra-red spectroscopy (NIRS) or the use of bioreductive markers such as 2-nitroimidazole derivatives belong to the former series of methods, while ^{19}F NMR spectroscopy/imaging, blood oxygen level dependent (BOLD) imaging, or electron paramagnetic resonance (EPR) and Dynamic nuclear polarization (DNP) using oxygen sensitive spin probes belong to the latter.[2] EPR methods have the advantage of the non invasiveness combined with a high sensitivity and specificity. They are mostly based on the broadening of the signal caused by Heisenberg exchange between molecular oxygen and the spin probe to determine pO_2. Two different types of spin probes are used in EPR oximetry, either particulate materials like lithium phthalocyanine, chars, coals, carbon black, or soluble molecules, namely, nitroxides and the triarylmethyl (trityl) radicals. By the late 90s, Nycomed Innovation AB featured original Gomberg's trityl radical in order to avoid hydrogen hyperfine coupling and enhance its stability and water solubility.[3] A new family of trityl spin probes was synthesized, also known as tetrathiatriarylmethyl (TAM), bearing four sulfur atoms on the phenyl ring (Fig. 1). The most representative members are water soluble **CT-03**, **deuterated CT-03** and **OX063** which exhibit a very narrow EPR linewidth, non toxic properties and are less sensitive to bioreduction than nitroxides.[4,5] Due to their unique properties, TAM type radicals have found number of MR applications as oxygen/pH sensitive spin probes or as contrast agents in electron paramagnetic resonance imaging (EPRI) and Overhauser magnetic resonance imaging (OMRI).[5,6] Moreover, TAM radicals have also been used for measuring superoxide radical by EPR spectroscopy or by spectrophotometry.[7] Recently, creative efforts have been done for the synthesis of these complex molecules.[8,9] **CT-03** can now be synthesized in large-scale in an efficient way.[10]

Among useful solvents, perfluorinated ones are well known for their excellent capacity to dissolve a high quantity of non polar gases like O_2. Many of them can at physiological temperature

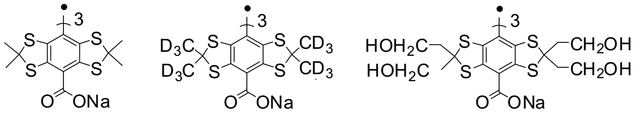
CT-03 **DeuteratedCT-03** **OX063**

Figure 1. Representative Nycomed's trityl radicals.

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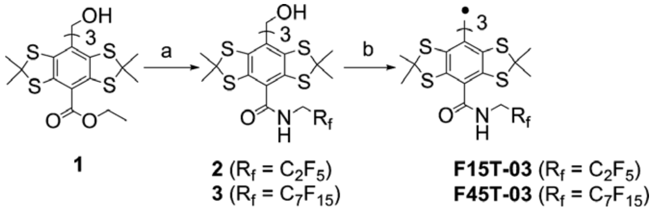
dissolve up to 40–50% *v/v* oxygen at 1 atm.[11] For instance, several PFCs have been used in vivo for their excellent oxygen solubility, such as hexafluorobenzene (HFB) that is utilized in animal models, and perfluorooctylbromide (PFOB) that is in clinical use. Recently, the group of Kuppusamy took advantage of that special property in the synthesis of a triethoxycarbonyl perchlorotriarylmethyl radical (PTM-TE) and the use of PTM-TE/HFB formulation for high-resolution oxygen mapping in tumor using EPR spectroscopy.[12,13] This group reported a high sensitivity of the line broadening with molecular oxygen in HFB. The sensitivity in HFB is at least 10 times as high as in DMSO, mainly due to the high solubility of oxygen in this solvent.

In the course of our research on the development of new tools for the assessment of tumor oxygenation by EPR spectroscopy using biocompatible perfluorocarbon (PFC) emulsions, we sought to enhance the affinity of the oxygen spin probe for a PFC formulation by attaching a perfluorinated tag on the trityl radical. Indeed, the radical derived from trityl ethyl ester **1** is not soluble in PFCs (solubility inferior to 0.1 mM in PFOB), so introducing a fluorous label on a molecule is the usual strategy to enhance its fluorophilicity.[14] Hereby we report a straightforward access to original fluorinated trityl oxygen probes.

Two new highly fluorinated TAM radicals, **F15T-03** (20% fluorine by weight) and **F45T-03** (40% fluorine by weight) were efficiently synthesized in a two-step sequence from precursor **1** as depicted in Scheme 1. Trityl ester **1** was obtained according to the procedure described in the literature.[8] The aminolyse of ethyl ester **1** by perfluoroamine ($H_2NCH_2R_f$) in presence of trimethylaluminum resulted in the formation of fluorinated trityl alcohols **2** ($R_f = C_2F_5$) and **3** ($R_f = C_7F_{15}$) in 95% and 66% yields, respectively, after purification by preparative thin layer chromatography (TLC).[15] It is noteworthy to mention the low nucleophilicity of these two commercially available fluoroamines. Nevertheless, our conditions allowed to reach good to excellent isolated yields. In this first step, the labile trityl alcohol was protected in situ by the formation of an aluminum alcoholate. Then, treatment of the trityl alcohols **2-3** with $BF_3.Et_2O$ gave the corresponding carbocations which are subsequently reduced by $SnCl_2$ to afford the persistent captodative radicals **F15T-03** and **F45T-03**.[16]



Scheme 1. Reagents and conditions: (a) $AlMe_3$, $H_2NCH_2R_f$, DCE, reflux, overnight (95%, $R_f = C_2F_5$; 66%, $R_f = C_7F_{15}$). (b) 1-$BF_3.Et_2O$, DCM, 1 h, 2-$SnCl_2$/THF, 30 min.

The EPR properties of the two new TAM-type radicals were comparable to those of other tetrathiatriarylmethyl radicals described previously, such as radical of compound **1**.[8] A single sharp peak was observed for **F15T-03** and **F45T-03**. The sensitivity of the EPR linewidth to oxygen was measured by carrying out the calibration of line broadening ΔB_{pp} (G) versus pO_2 (mmHg). Calibration curves were built in HFB and PFOB, for **F15T-03** and **F45T-03**, respectively, according to their respective high solubility in these solvents. **F15T-03** in HFB showed a single sharp peak with a linewidth of 0.550 G under anaerobic conditions and 3.55 G in ambient room air (21% oxygen) (Fig. 2). The slope of the calibration curve (18.7 mG/mmHg) is consistent with the results already published on the PTM-TE in HFB (Fig. 3).[12] **F45T-03** in PFOB showed a single sharp peak with a linewidth of 0.545 G under anaerobic conditions and 3.34 G in ambient room air with a slope of the calibration curve of 17.5 mG/mmHg. The sensitivity of compound **1** radical was not measurable using the same conditions due to its poor solubility in PFCs. For comparison, the sensitivity of **CT-03** in water was 0.64 mG/mmHg. Thus, our novel probes dissolved in PFCs are about 30-fold more sensitive. As mentioned previously the better sensitivity of line broadening to pO_2 in perfluorous liquids is the consequence of the better solubility of oxygen in such media. While water dissolves 3.1 vol% (25 °C) of oxygen, HFB is able to

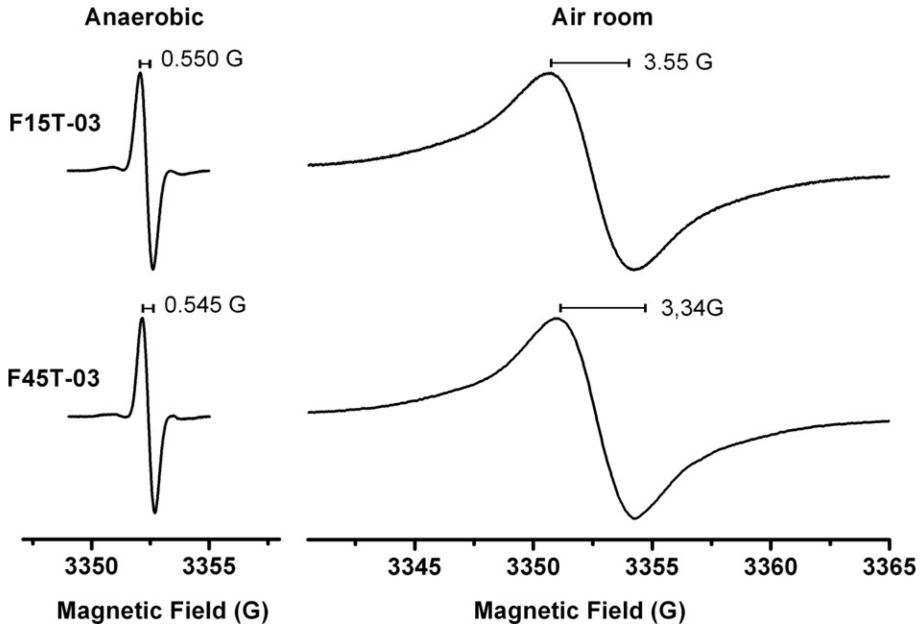


Figure 2. X-band EPR spectra, linewidths (ΔB_{pp}) of **F15T-03** in HFB, **F45T-03** in PFOB under anaerobic and air room conditions. The EPR acquisition settings were (a) **F15T-03** in HFB; 1 mM filtered on 0.2 μm (Pall Ghp Acrodisc); anaerobic conditions; sweep width: 10 G; power: 200 μW; temperature: 310 K; frequency modulation: 10 kHz; modulation amplitude: 0.15 G; time constant: 10.24 ms; conversion time: 10.24 ms. (b) F45T-03 in PFOB; 0.25 mM filtered on 0.2 μm (Pall Ghp Acrodisc); anaerobic conditions; sweep width: 6 G; power: 50 μW; temperature: 310 K; frequency modulation: 100 kHz; modulation amplitude: 0.1 G; time constant: 20.48 ms; conversion time: 20.48 ms.

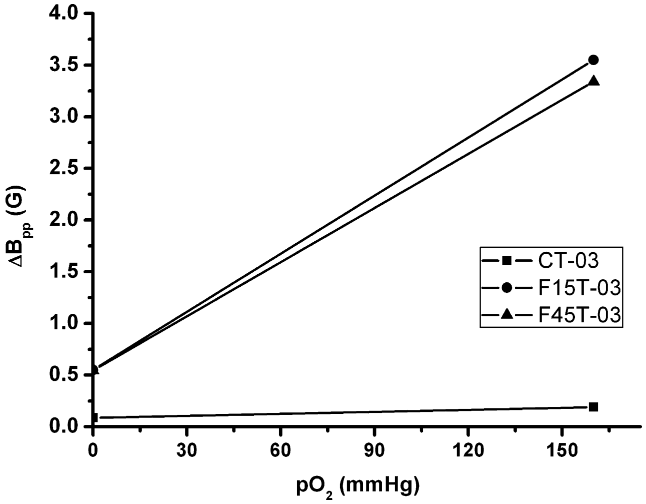


Figure 3. Calibration curves of linewidth ΔB_{pp} (G) as a function versus pO_2 (mmHg) for (■) **CT-03** (in water), (●) **F15T-03** (in HFB) and (▲) **F45T-03** (in PFOB).

dissolve 46.8–48.8 vol% (25 °C) and PFOB 50.0–52.7 vol% (25 °C) of molecular oxygen.[17]

Further development of the present study is to use these fluorinated tetrathiatriarylmethyl radicals as components of nanocapsules containing PFCs. These systems should present high sensitivity to oxygen and should be biocompatible and injectable to living systems.

In conclusion, we have disclosed an efficient synthesis of two new TAM-based radicals possessing a high affinity to fluorous media. These radicals are specially designed for assessment of tumor oxygenation using PFC formulations. Moreover, the sensitivity of line broadening is higher in PFC liquids than in water in agreement with the higher solubility of oxygen in these solvents.

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15. (a) *Preparation of **2**:* To a stirred solution of 2,2,3,3,3-pentafluoropropan-1-amine (68 mg, 0.454 mmol, 10 equiv) in dichloroethane (DCE) (1 mL) was slowly added at room temperature a 2 M solution of trimethylaluminum in toluene (0.227 mL, 0.454 mmol, 10 equiv). The mixture was stirred at room temperature for 15 min and trityl ester **1** (50 mg, 0.045 mmol, 1 equiv) in DCE (1 mL) was added. The mixture was stirred at room temperature for 1 h and refluxed overnight. After cooling to room temperature, the resulting orange mixture was diluted with dichloromethane (DCM) (5 mL) and carefully treated with saturated aqueous KH_2PO_4 (5 mL), and the organic layer was separated. The aqueous layer was extracted with DCM (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The orange residue was purified by preparative TLC (plate 20 × 20 cm, silicagel 60 F_{254}, 1 mm, Merck) using DCM to provide 60.8 mg (95%) of compound **2** as an orange solid. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.69 (s, 18H), 1.81 (s, 18H), 4.18 (m, 6H), 6.52 (s, 1H), 6.67 (t, $^3J = 6.1$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 27.5, 29.1, 32.2, 34.7, 39.2 (t, $^2J = 24$ Hz), 62.6, 63.2, 84.1, 113.0 (tq, $^1J = 253.3$ Hz, $^2J = 37.3$ Hz), 118.8 (qt, $^1J = 284.3$ Hz, $^2J = 34.7$ Hz), 125.6, 133.0, 137.6, 138.0, 140.3, 141.0, 167.1. ^{19}F NMR (282 MHz, $CDCl_3$) δ (ppm): −121.61 (s, 6F), −84.63 (s, 9F). ATR-IR (cm $^{-1}$): 3350, 2960, 2923, 2864, 1670, 1527, 1195, 1151. HRMS (TOF MS ES+) *m/z* $[M+Na]^+$ calcd for $NaC_{49}H_{46}F_{15}N_3O_4S_{12}$: 1431.9795, found 1431.9755. *Preparation* of **3**: The previous procedure was applied to the synthesis of **3** using a solution of 1H,1H-perfluorooctylamine (181 mg, 0.454 mmol, 10 equiv) treated with a 2 M solution of trimethylaluminum in toluene (0.227 mL, 0.454 mmol, 10 equiv) and then with trityl ester **1** (50 mg, 0.045 mmol, 1 equiv). The crude product was purified by preparative TLC (plate 20 × 20 cm, silica gel 60 F_{254}, 1 mm, Merck) using DCM/petroleum ether (2:1) to provide 65 mg (66%) of compound **3** as an orange solid. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.69 (s, 18H), 1.82 (s, 18H), 4.23 (m, 6H), 6.53 (s, 1H), 6.68 (t, $^3J = 6.3$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 27.6, 29.1, 32.2, 34.7, 39.6 (t, $^2J = 23.3$ Hz), 62.6, 63.3, 84.2, 100–125 (21C), 125.7, 133.1, 137.6, 138.0, 140.3, 141.0, 167.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ (ppm): −126.59 (s, 6F), −123.70 (s, 6F), −123.18 (s, 6F), −122.48 (s, 6F), −122.21 (s, 6F), −117.75 (s, 6F), −81.23 (s, 9F). ATR-IR (cm^{-1}): 3342, 2962, 2920, 2851, 1670, 1527, 1225, 1201, 1147. HRMS (ESI+) *m/z* $[M+1]^+$ calcd for $C_{64}H_{47}F_{45}N_3O_4S_{12}$: 2159.9491, found 2159.9451.; For an example of using trimethylaluminum for conversion of esters to amides, see: (b) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *18*, 4171.
16. *Preparation of **F15T-03**:* To a stirred solution of **2** (120 mg, 0.085 mmol, 1 equiv) in DCM (20 mL) was added dropwise at room temperature $BF_3.Et_2O$ (0.085 mL, 0.680 mmol, 8 equiv). After stirring for 1 h, the resulting dark solution was treated with a solution of $SnCl_2$ (27 mg, 0.145 mmol, 1.7 equiv) in THF (4 mL). The mixture was stirred at room temperature for 30 min. Saturated aqueous KH_2PO_4 (10 mL) was added and the organic layer was separated. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to provide 118 mg of the crude trityl radical **F15T-03** as a dark green solid. ATR-IR (cm^{-1}): 3335, 2960, 2918, 2849, 1670, 1524, 1196, 1151. HRMS (TOF MS ES+) *m/z* $[M]^{\cdot+}$ calcd for $C_{49}H_{45}F_{15}N_3O_3S_{12}^{\bullet}$: 1391.9870, found 1391.9854. *Preparation of **F45T-03**:* To a stirred solution of **3** (43 mg, 0.020 mmol, 1 equiv) in DCM (8 mL) was added dropwise at room temperature $BF_3.Et_2O$ (0.020 mL, 0.159 mmol, 8 equiv). After stirring for 1 h, the resulting dark solution was treated with a solution of $SnCl_2$ (6 mg, 0.034 mmol, 1.7 equiv) in THF (1 mL). The mixture was stirred at room temperature for 30 min and worked up as previously (**F15T-03**) to provide 43 mg of the crude trityl radical **F45T-03** as a dark green solid. ATR-IR (cm^{-1}): 3335, 2959, 2922, 2851, 1670, 1522, 1236, 1205, 1148. Cyclic voltammetry was carried out using about 0.5 mM of **F15T-03** and **F45T-03** in degassed DCM containing 0.1 M of tetrabutylammonium tetrafluoroborate. The redox potentials were measured with a glassy carbon working electrode, a platinum-foil counter electrode, and Ag/AgCl in EtOH LiCl saturated as the reference electrode. Scan rate was 50 mV/s. Similar cyclic voltammograms to the ester derivatives[9] were obtained with a $E(ox)_{1/2} = +\ 0.9$ V and $E(red)_{1/2} = -0.4$ V.
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