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

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Article info
Article history:
Received 25 March 2008
Revised 27 June 2008
Accepted 28 June 2008
Available online 3 July 2008

Keywords:
Ester aminolysis
Trityl radical
Oximetry
EPR
Tetrathiatriarylmethyl
TAM

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). Therefore, it is of particular importance for pO2 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, phosophorescence 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 19F 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. 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 pO2. 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 radicals in order to avoid hydrogen hyperfine coupling and enhance its stability and water solubility. 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. 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 (EPR) and Overhauser magnetic resonance imaging (OMRI). Moreover, TAM radicals have also been used for measuring superoxide radical by EPR spectroscopy or by spectrophotometry. Recently, creative efforts have been done for the synthesis of these complex molecules. CT-03 can now be synthesized in large-scale in an efficient way.

Among useful solvents, perfluorinated ones are well known for their excellent capacity to dissolve a high quantity of non polar gases like O2. Many of them can at physiological temperature...
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 per chlorotriarylmethyl 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 fluororous 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 (H2NCH2Rf) in presence of trimethylaluminate 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 BF3.Et2O gave the corresponding carbocations which are subsequently reduced by SnCl2 to afford the persistent captodative radicals F15T-03 and F45T-03.

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 ∆\(\Gamma_{pp}\) (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). 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 perfluorocarboxylic 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 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 per chlorotriarylmethyl 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.

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References

cial support of this work. J.M.-B. is senior research associate of the
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more oxygenation using PFC formulations. Moreover, the sensitivity
media. These radicals are specially designed for assessment of tu-
molecular oxygen.17

Figure 3. Calibration curves of linewidth $\Delta B_{\text{pp}}$ (G) as a function versus $p_{O_2}$ (mmHg)
for (B) CT-03 (in water), (C) FIST-03 (in HFB) and (A) 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
tetra(triarylmethyl)methyl radicals as components of nanocap-
sules 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 tu-

Acknowledgments

Université catholique de Louvain, FNRS, Fonds Joseph Maisin,
PAI 6/38 and ARC 04/09-317 are gratefully acknowledged for
financial support of this work. J.M.-B. is senior research associate of
the Belgian FRS-F.N.R.S (Fond National de la Recherche Scientifique,
Belgium).

References

1. (a) Antioxidants: In Science, Technology, Medicine and Nutrition; Scott, G., Ed.;
Albion Publishing: Chichester, 1997; (b) Radicu libres et stress oxydant
Aspects aspects biologiques et pathologiques; Delattre, J., Beaudeux, J.-L.,
(b) Andsersen, S.; Radr, F.; Rydbeck, A.; Servin, R.; Wistrand, L. G. U. S. Patent 5330140, 1996.;
(c) Ardenkjaer-Larsen, J. H.; Leunbach, I. PCT Int. Appl. w0907063, 1997.;
(d) Andsersen, S.; Radner, F.; Rydbek, A.; Servin, R.; Wistrand, L. G.;
Jorgensen, M.; Rise, F.; Andersson, S.; Almen, T.; Aabye, A.; Wistrand, L. G.;
J. Magn. Reson. Med. 2000, 43, 634; (b) Bobko, A. A.; Dhimitruka, I.; Zweier, J. L.; Khramtsov, V. V.
(d) Subramanian, S.; Yamada, K.-I.; Irie, A.; Murugesan, R.; Cook, J. A.;
Med. 2002, 47, 1001; (e) Li, H.; Deng, Y.; He, G.; Kuppusamy, P.; Liu, D. J.;
Matsumoto, K.-I.; Subramanian, S.; Devasaahayam, N.; Aravalluvan, T.;
Med. 2006, 55, 1157.
5. (a) Cazzaniga, A.; Kumar Katala, V.; Parandini, N. L.; Zweier, J. L.;
Kuppusamy, P. Free Radical Biol. Med. 2003, 35, 602; (b) Katala, V.;
81.
6. (a) Xia, S.; Villamen, F. A.; Hadad, C. M.; Kuppusamy, P.; Li; Y.; Zhu, H.; Zweier,
2008, 73, 1490.
8. Dhimitruka, I.; Velayutham, M.; Bobko, A. A.; Khramtsov, V. V.; Villaman, F. A.;
9. Dhimitruka, I.; Velayutham, M.; Bobko, A. A.; Khramtsov, V. V.; Villaman, F. A.;