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RGD-conjugated triarylmethyl radical as probe for electron paramagnetic imaging

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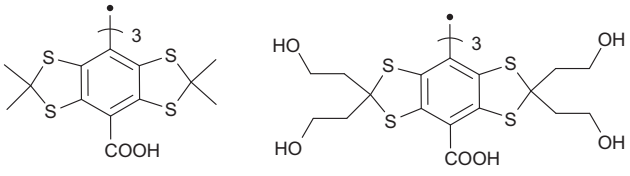
ABSTRACT

A stable RGD-conjugated triarylmethyl (TAM) radical **3** has been synthesized in a straightforward three-step sequence. **CT-03** 'the Finland trityl' which is a very popular contrast agent for electron paramagnetic resonance imaging has been covalently bound to the NH_2-ending of a pentapeptide commonly used to target integrins and confers tissue selectivity. Moreover, this new TAM radical is stable and sensitive to molecular oxygen.

Low frequency electron paramagnetic resonance (EPR) imaging is a technique that allows in vivo mapping of the distribution of a paramagnetic species, and other physiological parameters such as oxygen concentration, pH and redox status.[1,2] The physical basis of EPR is similar to the well known nuclear magnetic resonance (NMR). However, the magnetic moment of the electron is 658 times greater than that of the proton, which makes EPR intrinsically more sensitive than NMR for the same probe concentration. By contrast with magnetic resonance imaging (MRI) by NMR, there is no naturally occurring paramagnetic species in sufficient concentration and long-enough half-life to be imaged in vivo by EPR imaging (EPRI), with the exception of melanin.[3,4] Thus an exogenous paramagnetic spin probe is needed. Among the water soluble radicals used in EPRI, the triarylmethyl (TAM) radicals bearing four sulfur atoms on each phenyl ring such as **CT-03** and **Ox063** (Fig. 1) are the most attractive.[5]

These compounds initially developed to be used as hyperpolarizing agents for Overhauser-enhanced magnetic resonance imaging (OMRI)[6] exhibit a good stability, a single narrow EPR line of less than 100 mG under anoxic conditions (0% oxygen), a low concentration-dependent line-broadening and non toxic properties.[5] Indeed, a narrow EPR line confers high sensitivity of detection, particularly useful in vivo, since the intensity of the signal is inversely proportional to the square of the line width. Yet, the synthesis of such complex molecules remains challenging and new structures are regularly reported. Original Nycomed's TAMs and subsequent chemically modified TAMs have been used for EPR measurements of intracellular[7] and extracellular[8] O_2 concentrations, pH,[9,10] thiol,[11] superoxide anion,[12] redox status,[13] spin–spin interdistance,[14] for EPRI[15] and employed as hyperpolarizing agents.[6] However, to date, only triarylmethyl radicals with unspecific distribution have been reported.

Hereby, we describe a straightforward synthesis of the pentafluorophenol ester derivative **2** of **CT-03**. This activated form of **CT-03** can be used to bind the trityl core to a targeting moiety designed to achieve tissue selectivity. Compound **2** has been used to conjugate the TAM radical to the pentapeptide GRGDS (Gly-Arg-Gly-Asp-Ser) including the RGD sequence, known to bind some of the trans-membrane proteins called integrins which are commonly used as targets for molecular imaging.[16]



CT-03 OX063

Figure 1. Chemical structure of **CT-03** and **Ox063**.

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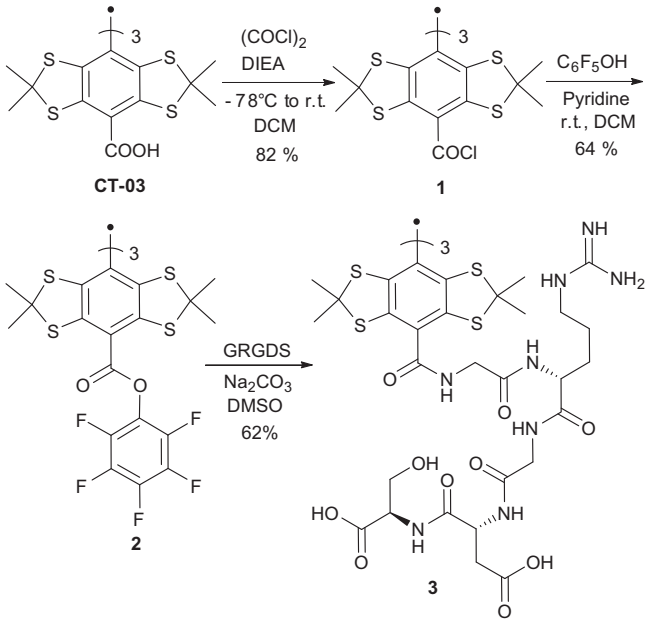

Scheme 1. Synthesis of **3**.

The synthesis has been achieved as depicted in Scheme 1. **CT-03** is synthesized according to a procedure described in the literature.[17] Activation of the carboxylic acid functions of **CT-03** by oxalyl chloride and diisopropylethylamine (DIEA) leads to the formation of **1** in 83% yield after filtration on a small silica gel path. Then, esterification of **1** with pentafluorophenol and pyridine furnishes **2** which is stable enough to be purified by column chromatography. This compound was recovered in a pure form in 64% yield.

Compound **2** is an activated form of **CT-03** and can be used to conjugate the TAM radical to any carrier device. In order to prove the concept, **2** was mixed in DMSO with a small excess of unprotected pentapeptide GRGDS in the presence of sodium carbonate. Triarylmethyl radical **3** coupled to three GRGDS peptides, most probably through the reactive terminal NH_2 function, was formed with more than 90% conversion. Purification on semi-preparative HPLC carried out on reverse-phase gives **3** in a pure form (Fig. 2a) in 62% yield. While only one peak is visible on the chromatogram obtained under gradient elution (Fig. 2a), two peaks with the same areas are obtained under isocratic elution (Fig. 2b). These two peaks correspond to diasteroisomers associated with the helicoidal chirality of the trityl core.[18,19]

Indeed, the trityl core displays a propeller conformation where all rings have the same direction of twist. Both right-handed (*P*) and left-handed (*M*) propeller conformations exist and display enantiomeric relationship. We have previously shown that for the tetrathiatriarylmethyl core, the isomerization between the two enantiomeric conformations is slow, which confers chiral properties to these molecules.[18,19] As GRGDS is an enantiomerically pure molecule, two diastereoisomers are obtained for the conjugated compound **3**.

The EPR spectrum of **3** has been recorded at X-band in phosphate buffer saline (PBS) (Fig. 3). The spectrum shows a single EPR line with a peak-to-peak linewidth ΔB_{pp} = 705 mG under air room conditions (21% O_2). The wider linewidth obtained for **3** than for **CT-03** can be explained by unresolved hyperfine splitting with nitrogen atoms of the amide functions. This observation has been already reported for dendritic TAM radicals.[20] Bimolecular collision between a TAM radical and molecular oxygen induces a broadening of the EPR line of the TAM spin probe. This phenomenon can be used to assess oxygen concentration by EPR. According to the Smoluchowski equation there is a linear relationship between the linewidth and O_2 concentration for a soluble spin probe. With 0% of oxygen, the linewidth of **3** reaches ΔB_{pp} = 630 mG, indicating that compound **3** keeps its sensitivity to oxygen and can be used as an EPR oxymetric probe (sensitivity: 0.47 mG/mmHg).

Recent studies about in vitro metabolism of **CT-03** and **Ox063** have shown that they could be reduced under anaerobic conditions in the presence of liver microsomes to the corresponding triarylmethane through formation of the anion.[21] Furthermore, a TAM cation is obtained through oxidation of radicals by peroxidases and related hemoproteins.[22] The cation can subsequently react with nucleophiles such as water, peroxides, phosphines, thiols, amines, etc. The redox potential of compound **3** has been measured in PBS (pH 7.4) by cyclic voltammetry (CV) (Fig. 4) and compared with **CT-03**.

The results show that compound **3** can be reduced more easily than **CT-03** but is harder to oxidize than **CT-03** (Table 1). This can be explained by the higher electron-attracting power of amide functions of **3** by comparison with the carboxylate functions of **CT-03** in aqueous medium. Thus, radical **3** appears to be more stable than **CT-03** under oxidation conditions.

In conclusion, we have synthesized for the first time a tetrathiatriarylmethyl radical linked to three carrier molecules. Compound **3** has been synthesized by mixing pentafluorophenol ester **2** with an unprotected pentapeptide sequence, followed by purification

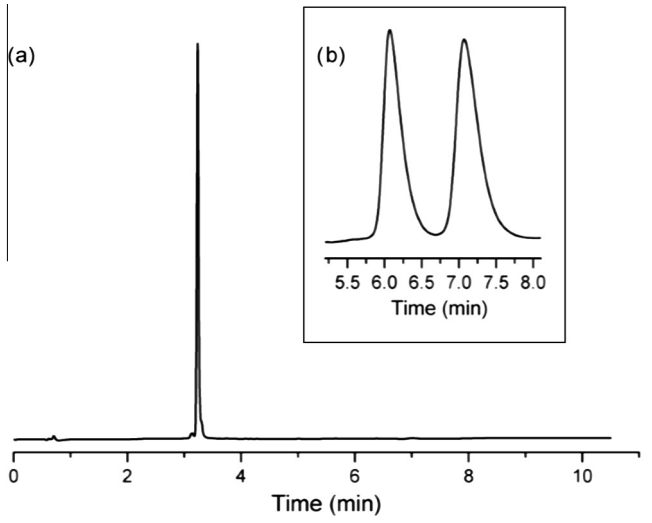

Figure 2. HPLC chromatograms of **3** under gradient (a) and isocratic (b) elution.

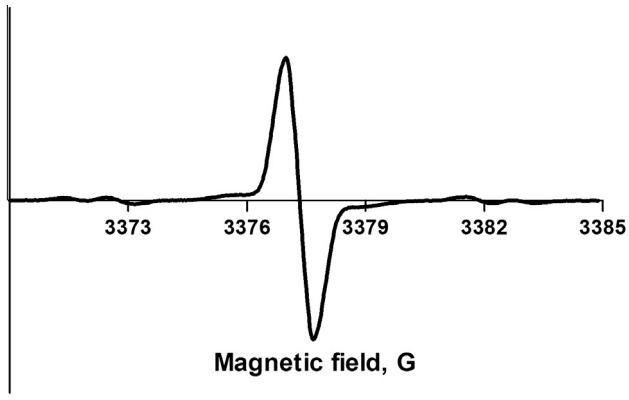

Figure 3. EPR spectrum of **3** recorded in PBS (pH 7.4) at X-band.

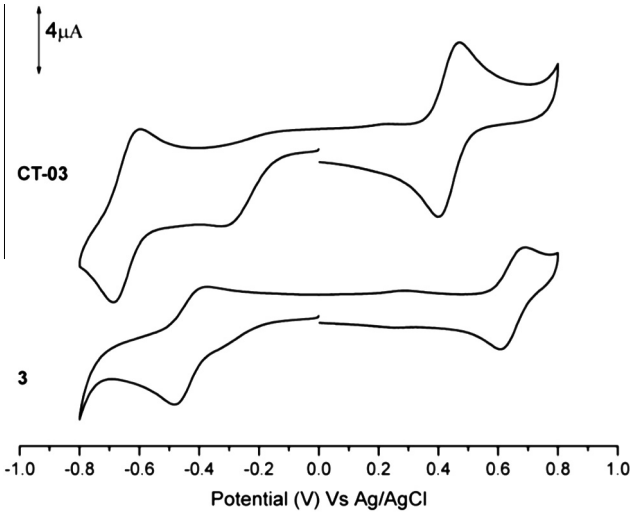

Figure 4. Cyclic voltammogram of **CT-03** and **3**.

Table 1
Redox potentials ($E_{1/2}$) relative to Ag/AgCl (KCl 3 M) determined by CV

Compound	Solvent	$E_{1/2}$ ox[a]	$E_{1/2}$ red[a]
CT-03	PBS	+0.434	−0.642
3	PBS	+0.648	−0.429

[a] Calculated according to $E_{1/2} = (E_{pa} + E_{pc})/2$.

by semi-preparative HPLC. The RGD-conjugated TAM **3** keeps the sensitivity to oxygen. Its oxidation potential is higher than that of **CT-03** while its reduction potential is lower. Thus radical **3** could be considered as a potential spin label possessing tissue selectivity for EPR O_2 measurements and for EPRI. Moreover, the activated ester **2** is a valuable key-intermediate which can be used to bind other carrier molecules to the TAM radical.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.08.120.

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